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From the Department of Cytology, Warsaw University

OOGENESIS IN MIKIOLA FAGI HART. (CECIDOMYIIDAE; DIPTERA)*

By

BOHDAN MATUSZEWSKI

With 114 Figures in the Text

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Contents

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A. Introduction

Previous studies on the cytology of the *Cecidomyiidae* (see WHITE 1954) have revealed them to be, along with the *Sciaridae* (METZ 1938) and the *Orthocladiinae* (BAUER and BEERMANN 1952), a group of insects of special interest for the study of chromosome cycle evolution.

All species of the *Cecidomyiidae* examined so far possess in germ cells more chromosomes than in somatic ones. The difference is due to the elimination from the nuclei of future somatic cells of a certain number of E-chromosomes, characteristic of the given species. This elimination occurs during the early cleavage divisions. In the nuclei of somatic cells only the S-chromosomes that divide in the regular way are retained. Contrary to the nuclei of somatic cells, divisions of the nuclei of primordial germ cells are quite normal, these nuclei and their derivatives (oogonia, spermatogonia) contain the S- and the E-chromosomes.

Until recently most of the information on the oogenesis of the *Cecidomyiidae* came from studies of the paedogenetic development of *Miastor metraloas* (KAHLE 1908; KRACZKIEWICZ 1935, 1936a; WHITE 1946) and *Oligarces paradoxus* (REITBERGER 1940, HAUSCHTECK 1959).

Oogenesis in *Phytophaga destructor*, a representative of a bisexual species, was described by METCALFE (1935), but in the light of later studies (KRACZKIEWICZ 1938, 1950; WHITE 1946, 1947a and b, 1950) her description seems to contain some misleading statements, serious enough to make it of rather little value for the understanding of how the sexual oogenesis in *Cecidomyiidae* proceeds. Later, some prophase stages of sexual oogenesis in *Miastor metraloas* (KRACZKIEWICZ 1936b, 1937) and in a number of species of the subfamily *Cecidomyiinae* (WHITE 1950) were described. WHITE, when studying the diakinesis and prometaphase stages, found that the behaviour of S-chromosomes differs from that of E-chromosomes. Thus, chiasmatic bivalents were formed by S-chromosomes only, while E-chromosomes in all of the species investigated always occurred as univalents. WHITE failed to observe both the early prophase stages and maturation divisions of the eggs.

On the basis of his observations WHITE advanced a hypothesis (1950) which assumed that sexual eggs of *Cecidomyiidae* undergo two maturation divisions, the number of the S-chromosomes being reduced, while the univalent E-chromosomes undergo two equational divisions. WHITE did not reject the possibility of a single division of E-chromosomes, but he made so suggestion as to the course of meiosis. In the latter case, of course, the pattern of oogenesis would be still more complicated.

The aim of the present work was to gain as deep an insight as possible into the sexual oogenesis in *Cecidomyiinae*, which could provide a confirmation of WHITE's above mentioned hypothesis and fill the still

existing gap in the general scheme of the chromsome cycle in this subfamily.

The author wishes to express his deepest thanks to Prof. ZYGMUNT KRACZKIEWICZ for suggesting this investigation as well as for his helpful discussions in the course of this work.

B. Material and Methods

In Poland *Mikiola fagi* HART. usually occurs abundantly in all larger stands of beech trees. As a result of its secretion of growth substances (BOYSEN JENSEN 1948 1952) the larva of this gall midge produces a gall on the upper side of beech leaves. A fully developed gall has the form of a pointed cone, having smooth, hard, green walls, turning red towards the end of the summer. Usually only one larva is present in a gall, however two larvae can also be found, although rather rarely.

In the second half of September, or in October, shortly before metamorphosis of the larva into a pupa, the canal connecting the gall with the leaf becomes closed by a white membrane, which most probably consists of salivary gland secretion of the larva. Simultaneously, at the bottom of the gall, in the tissue of the leaf, a separation layer is produced, which facilitates the separation of the gall from the leaf.

M. fagi hibernate in their galls as pupae. Adults leave the galls at the end of March or in April. Eggs, which will later hatch into the single annual generation of *M. fagi* are laid by females on leaf buds.

The material used for the investigations had been collected in wooded valleys of the Polish Tatra in the vicinity of Zakopane.

Larvae intended for studying divisions of somatic and oogonial cells were collected together with their galls, in the summer time, from July to October. To obtain pupae, containing oocytes in successive stages of development, the following procedure was applied.

Beech leaves with attached galls were collected at the end of September, placed in large vessels, containing discs of moist blotting paper covered with glass plates. In time the galls fell off from the leaves and were then placed in Petri dishes covered inside with moist blotting paper. The galls in the dishes, which had been kept outdoors until frost, were then stored at a temperature of 0 to —2° C in a refrigerator. It had been noticed in the course of several successive years that under stable thermal conditions imagines usually emerge from the puparium in the second half of April, the process usually lasting a relatively short period of time, namely 4—7 days. Lowering the storage temperature to 3—4 degrees below zero shortly before the adults emerge, inhibits the above process. This phenomenon, however, has proved to be reversible. Due to this fact it was possible to obtain, in the period from middle of April to middle of June, any required number of female imagos. To this end only a number of galls had to be transferred from the refrigerator to a large glass dish covered with a glass plate and placed outside the window. The following morning either newly emerged imagines were found in the glass dish or the eclosion process itself could be observed. At room temperature females start egg laying within 1—1.5 hours after emerging.

Larvae, and later pupae, were fixed in batches of 10—15 of individuals at several days intervals in the period of time from autumn till next spring, when adults started to appear. Adult females were fixed immediately after emerging as well as before and during egg deposition.

The material used for the studies was fixed in Bauer's modification of Bouin-Allen. Larvae and young pupae after having been pierced with needles were fixed as a whole; of older pupae and of adults only ovaries were fixed. Eggs laid on

moist blotting paper in a small dish were fixed in the fixative heated to 60° C, at various time intervals starting from the beginning of egg laying. The eggs of *M. fagi* are small (0.3 mm long and 0.09 mm wide), and the laying process is slow. It lasts several hours and cannot be accelerated by the methods given by BAYREUTHER (1956) for crane-flies. For this reason batches of up to several hundred eggs, laid within a certain period of time, were usually fixed, most of the eggs being in nearly the same stage of oogenesis.

The material was sectioned at 10—15 μ and stained in Heidenhain's iron hematoxylin, gentian violet or by the Feulgen method.

C. Observations

I. Somatic chromosomes of the female

The complement of S-chromosomes of females was examined in larval neuroblasts, as well as in somatic cells forming the anterior part

Fig. 1a—d. Female somatic metaphases. a, b and c from follicular cells. c, d from neuroblasts. a Gentian violet. b—d Hematoxylin

of the larval ovary. Divisions of these cells frequently occur during the whole period of larval life. Eight chromosomes were found in all metaphase plates examined (Figs. 1 and 2).

On the basis of relative sizes and kinetochore positions, in the best fixed metaphase plates (Fig. 1 a) one pair of large mediokinetic, one pair of large submediokinetic, and two pairs of small submediokinetic chromosomes can be distinguished among the four pairs of the S-chromosomes.

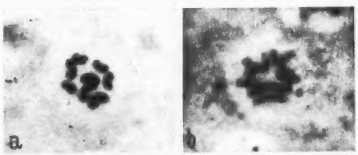


Fig. 2a and b. Female somatic metaphases. a from a neuroblast; b from a follicular cell Gentian violet. ×2300

These size differences of the mitotic chromosomes of the S-set in the cells examined, correspond to the size differences between the polytene chromosomes in the reservoir part of the salivary gland of *Mikiola fagi* (KRACZKIEWICZ and MATUSZEWSKI 1958). In the latter case they are more pronounced.

II. The germ-line chromosomes

The spherical larval ovary of *Mikiola fagi* consists of two distinctly delimited parts which differ from each other both in their size and in the stainability of their cells. The oogonia form a small aggregate

which occupies the smaller, posterior part of the ovary, while the much larger, anterior part is occupied by small, closely packed somatic cells, the follicular cells with a strongly basophilic cytoplasm. An analogous structure of ovaries has been found in a number of the cecidomyiid species (WHITE 1947a, 1950; KRACZKIEWICZ 1950).

The oogonia contain rather large vesicular nuclei, in which at interphase groups of eight heteropycnotic chromosomes can be observed (Fig. 3). Such a group of chromosomes is located just under the nuclear membrane, usually on one side of the nucleus. The chromosomes of this group occur as separate, spherical or slightly elongated and deeply

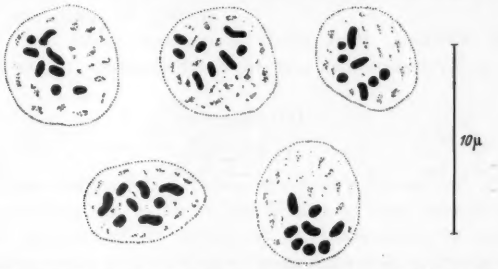


Fig. 3. Oogonial nuclei. In each nucleus a group of eight heteropycnotic S-chromosomes is seen. Hematoxylin

staining bodies. The rest of the germ-line chromosomes are represented by diffuse chromatin during the interphase period. A part from the heteropycnotic chromosomes, granules of rather faintly stained chromatin are scattered here and there within the nucleus, but even a rough estimate as to the number of the rest of chromosomes is impossible. On the basis of the coincidence of the number of S-chromosomes in somatic cells with the number of heteropycnotic elements in germ-line cells of the species, as well as on the basis of studies on cecidomyiid spermatogenesis (WHITE 1947a, 1950; KRACZKIEWICZ 1950) the assumption is made that in the interphase stage a set of S-chromosomes in oogonia is represented by a group of heteropycnotic chromosomes.

In the heteropycnotic chromosome groups present in oogonia of *Mikiola fagi* two pairs (or at least one pair) of larger elements can be distinguished which, most probably, correspond to two pairs of large chromosomes belonging to the S-set of somatic cells.

Although oogonia can undergo divisions during the whole period of larval life, the divisions occur rather rarely and are not synchronized. Shortly before metamorphosis of larvae into pupae oogonial divisions occur more frequently.

The number of chromosomes in metaphase plates of oogonial nuclei of *M. fagi* is 24, thus being identical with the number of germ-line chromosomes in *Trishormomyia helianthi* (WHITE 1950).

Among germ-line chromosomes both mediokinetic and submediokinetic chromosomes can be distinguished, yet all of them are of nearly the same size (Fig. 4). The S-chromosomes and the E-chromosomes show rather insignificant morphological differences, and, in addition, the degree of their condensation and the ability to stain are much the same. In addition, their small dimensions make the distinction between 8 S-chromosomes and 16 E-chromosomes rather impossible.

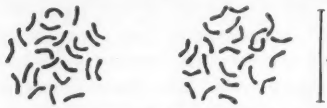


Fig. 4. Oogonial metaphases. Each plate contains 24 chromosomes. Gentian violet

III. Oogenesis

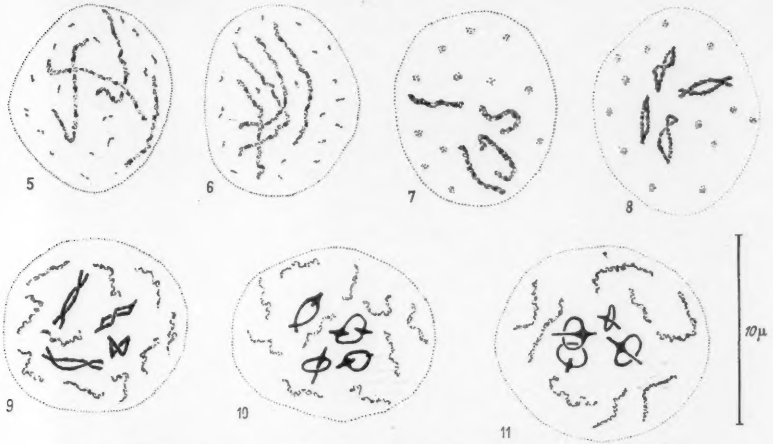
1. Prophase

a) The first period of the oocyte growth. The earliest stage of oocyte growth observed may be named pachytene on the ground of the first appearance of chromosomes in the nuclei of the oocytes. In *Mikiola fagi* the presence in a nucleus of only four long chromosomes which have no longitudinal splits is characteristic of this stage. The configuration of the above elements is mostly irregular (Figs. 5 and 12), sometimes, however, a more regular configuration can be observed (Fig. 6). Along the chromosomes, numerous brightly stained, short blocks of heterochromatin can be seen. The chromosomes differ little one from another, in position of heterochromatin, and length. For this reason a characterization of them and their identification in the nuclei of various oocytes seems rather impossible.

All the rest of the germ-cell chromosomes are during this period in a diffuse state, and the occurrence of diffuse, barely stainable granules of chromatin in the nuclear sap is the only indication of their presence. Later investigations have proved the four observed chromosomes to be bivalents. Thus, it should be assumed, as WHITE did (1950) for the bivalents observed in several cecidomyiid species during prophase and prometaphase stages of oogenesis, that the pachytene threads (bivalents) occuring in *M. fagi* at early prophase result from the conjugation between homologous S-chromosomes.

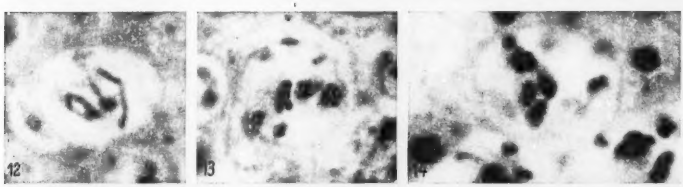
The length of pachytene chromosomes is gradually reduced (Fig. 7), their condensation increases rapidly, and they soon become intensely stainable, compact elements. Now, for the first time, splits between the conjugated S-chromosomes can be observed (Figs. 13 and 14).

Presently, in the cytoplasm of oocytes one or two spherical Feulgen negative bodies, which can readily be stained in gentian violet or hematoxylin, can be seen (Fig. 14).



Figs. 5—11. Early prophase stages. Figs. 5 and 6. Early pachytene. Fig. 7. Late pachytene. Figs. 8 and 9. Early diplotene. Figs. 10 and 11. Late diplotene. Hematoxylin

The slow separation of homologous chromosomes at the beginning of diplotene is accompanied by a lengthening of the chromosomes. These two processes lead to the formation of typical diplotene figures



Figs. 12—14. Early prophase stages. Fig. 12. Early pachytene. Figs. 13 and 14. Early diplotene. Hematoxylin. Figs. 12 and 14. ×2750. Fig. 13. ×2250

(Figs. 8 and 9), the appearance of which indicates the chiasmatic character of bivalents in *M. fagi*. The position of the bivalents, both inside the nucleus and towards one another, shows at this stage no regularity. The outlines of bivalents, initially "rough", gradually become "smooth"; simultaneously, repulsion of homologous chromosomes leads to pronounced opening out of bivalents between chiasmata and to the spreading of their free, chiasma-distal arms (Figs. 10 and 11). At the

same time the bivalents congregate. They finally take up a position as a group of four bivalents (Fig. 11) away from the rest of chromosomes which are only just becoming visible.

In the diplotene stage each of the four bivalents possesses at least two chiasmata. Because of the median of submedian positions of kinetochores in the bivalents in *M. fagi*, one can infer from the locations of the chiasmata that they are present in both chromosome arms, at each side of the kinetochore; their distances from the kinetochores seem, however, to vary in different bivalents.

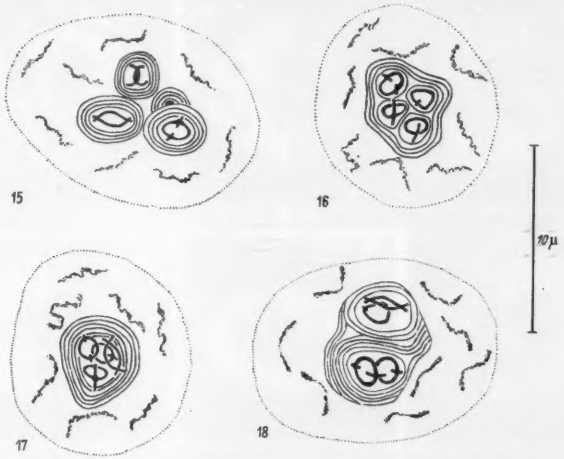
In Fig. 10 one, and in Fig. 11 two bivalents can be seen, with a more proximal position of one of the two chiasmata. In the other bivalents of these nuclei the chiasmata are more or less distal. In some of the bivalents three chiasmata seem to be present (Figs. 8 and 9). It may, however, be possible that some of these configurations do not represent chiasmata, but result either from overlapping of chromosomes which are not lying parallel to each other or from twists of interchiasmatic regions. On the other hand, in a number of cases analysis of considerably more advanced stages has definitely proved the presence of one bivalent, having one proximal and two distal chiasmata (Figs. 46 and 48). Taking into account both possibilities, the assumption seems reasonable, that in the oogenesis in *M. fagi* the number of chiasmata per oocyte is 8 or 9. Both the above observations and the further course of prophase indicate that chiasma-formation in *M. fagi* can considerably vary, similar to that in *Oligotrophus pattersoni*, as observed by WHITE (1950).

During the period of grouping of the bivalents inside the nucleus, a new, hitherto not observed nuclear component appears. This component can best be seen in slides deeply stained with iron hematoxylin: a layer of Feulgen negative substance begins to be formed on the surface of the bivalents. Of all of the dyes used, only hematoxylin stains this substance. Initially, when the bivalents are still at some distance from one another, the substance which because of its location is called perichromosomal surrounds each of the bivalents separately in a layer of nearly the same thickness (Fig. 19). Already at this time very faint concentric continuous lines can be seen in it, which may be regarded either as its fibrillar components or as an evidence of its lamellar structure (Fig. 15).

As far as these lines in the sectioned perichromosomal substance are concerned their artifact character is not quite impossible. They might for example have been formed as a result of strain in the gel-like substance under the influence of the fixative which had penetrated into the substance. They do not seem, however, to be any fissures or scratches made during embedding or sectioning, the slide showing proper fixation of the cells and especially of the nuclei. There is no doubt, however, as to the regularity in the occurrence of this perichromosomal substance, which should not be considered as a structure of artifact character, which has

been formed under the action of the fixative. The above statement has been confirmed by investigations on living material, now in progress.

After the bivalents have approached one another close enough to bring their perichromosomal layers into contact, the latter join to form a common coat around all the bivalent S-chromosomes in the middle of the nucleus. At first the complexity of the new structure can still be seen either from its irregular shape (Figs. 16 and 18) or from border lines between bivalents coated with perichromosomal substance (Figs. 22 and



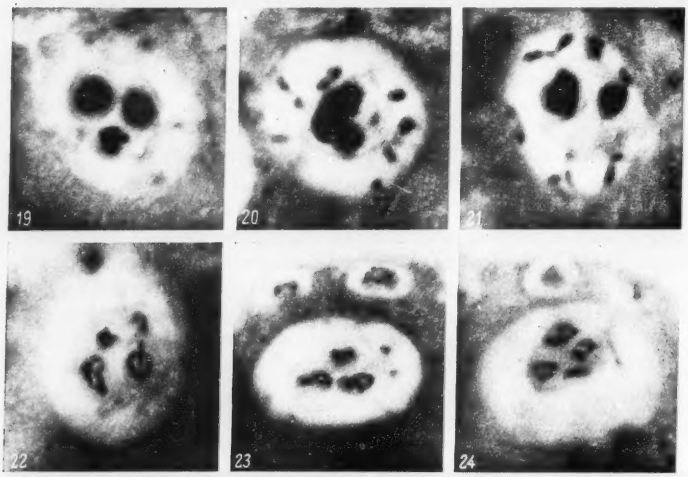
Figs. 15—18. Diplotene. Lamellar structure of perichromosomal substance before and after formation of inner part of nucleus. Hematoxylin

23). Sooner or later the above features gradually disappear, and in the end all the bivalents seem to be immersed in a mass of perichromosomal substance (Figs. 17, 20, and 21).

The grouping of the bivalents in the middle of the nucleus, involving the fusion of their perichromosomal layers, is the first step of the process of separation of the inner part of the nucleus, the existence of this part during oogenesis in *M. fagi* being a very distinctive, specific feature of the further prophase and prometaphase stages.

The process of differentiation of the E-chromosomes starts after the S-chromosomes have reached the early diplotene stage. The appearance of strongly staining granules in the nuclear sap is the first sign of this process. The granules are most probably heterochromatin parts of the E-chromosomes (Figs. 13 and 14). Soon, thin, faint chromosome threads become visible. The threads are initially rather short, strongly

twisted and uniformly distributed throughout the whole nucleus (Figs. 9 and 10). As the grouping of the bivalents proceeds, the E-chromosomes move towards peripheral parts of the nucleus (Figs. 11 and 20). This is accompanied by a gradual lengthening of the E-chromosomes and their increasing stainability. At the moment when the central part of the nucleus containing the bivalents has differentiated, the E-chromosomes appear as long threads (Figs. 30 and 31), which then continue to elongate



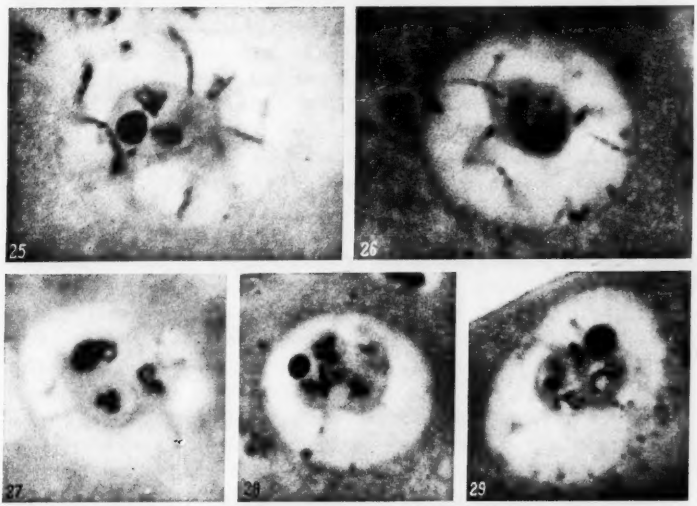
Figs. 19—24. Diplotene. Successive stages of formation of the inner part of nucleus. The lamellar structure of the perichromosomal substance disappears and the inner part of the nucleus becomes homogeneous and stains diffusively. See text for details. Hematoxylin. Figs. 19—21 ×2740; Figs. 22—24 ×2250

until a maximum is attained (Fig. 25, 26, 32, and 33). Thus, at the time when the bivalent S-chromosomes are in the late diplotene stage, the E-chromosomes hardly differ in their appearance from typical chromosomes at early mitotic prophase.

The apparent lengthening of the E-chromosomes, most probably is not a growth but a differentiation process, which starts at certain points and then gradually spreads over other, more distant parts. As it can often be seen at the initial period of condensation of the E-chromosomes, the contracted middle part of a chromosome is relatively poorly stained, in contrast to the short arms located symmetrically at either side of it (Fig. 18 and 21). This fact suggests that the kinetochores might be the sites from which condensation of the E-chromosomes starts. It is, however, not excluded that the sites of differentiation bear no relationship with the kinetochores, their locations being different in various E-chromosomes.

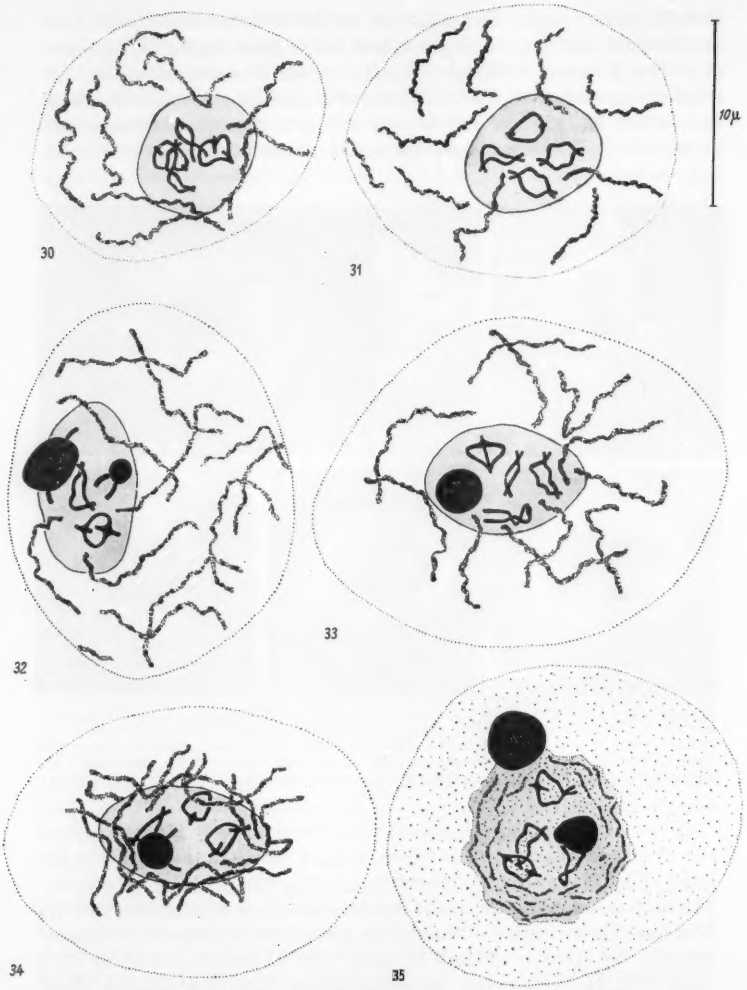
Neither during nor after differentiation do the E-chromosomes exhibit any tendency towards conjugation or loose association of the

somatic pairing type. The structure of the E-chromosomes also does not indicate that any conjugation had taken place during early stages of oocyte growth. Although counting of the E-chromosomes in this stage of oogenesis is very difficult, their number was several times found to be about 16. Thus, it may be assumed, that at prophase of oogenesis in *M. fagi* the E-chromosomes are univalent from the moment of their appearance.



Figs. 25—29. Diplotene. Production of nucleoli by S-chromosomes. Against the background of the diffusively staining inner part of the nucleus bivalents and nucleoli are visible. Only in Fig. 27 the lamellar structure of the inner part of the nucleus is still visible. Figs. 25—26. In the outer part of the nucleus univalent E-chromosomes are distinctly visible. Figs. 25 and 27: gentian violet. Figs. 26, 28 and 29: hematoxylin. ×2250

Towards the end of the period of differentiation of E-chromosomes the inner part of a nucleus is either situated nearly in the middle of the latter (Figs. 31 and 33) or shifted towards its periphery (Figs. 30 and 32). This shift seems, however, not to be related to the position of the nutritive chamber. The inner part of the nucleus now takes the shape of an ellipsoid and can be diffusely stained by such dyes as gentian violet or light green (Fig. 24). Sometimes the occurrence of fibrillar or lamellar structures (Fig. 27) can still be observed in this stage. Soon, for the first time since the beginning of prophase, nucleoli appear. They are produced in the inner part of the nucleus. Doubtlessly the bivalent S-chromosomes are responsible for their production. The figures, as the one shown in Fig. 32, indicate that at least two bivalents are nucleolus-producers.



Figs. 30—35. Diplotene. Position of inner part of nucleus and of condensing E-chromosomes in relation to each other before (Figs. 30—31) and soon after the beginning of nucleoli formation by the S-chromosomes (Figs. 32—33). Fig. 34. E-chromosomes, originally equally distributed in outer part of nucleus, now come into contact with the surface of the inner part of the nucleus. Fig. 35. E-chromosomes after inclusion in inner part of nucleus gradually lose their stainability. No perceptible changes occur in the appearance of S-chromosomes at this time. Figs. 30—31 and 34—35: hematoxylin. Figs. 32—33: gentian violet

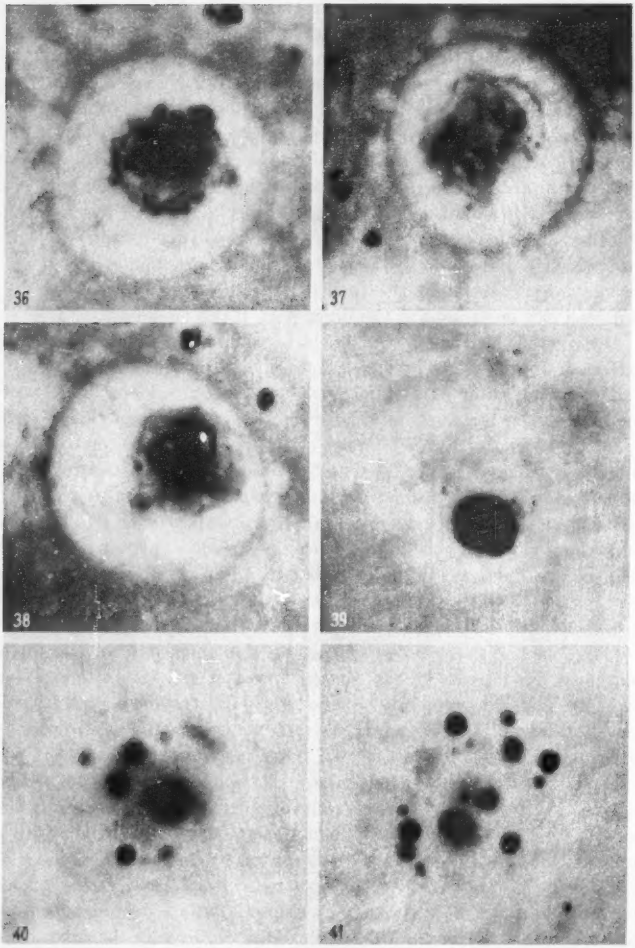
After the nucleoli have attained a certain size, their contact with the bivalents is broken. They then remain either completely imbedded in the substance constituting the inner part of the nucleus (Figs. 25, 26, and 33) or protrude more or less from it (Figs. 28, 29, and 32). Although such a behaviour of the nucleoli might be an indication of their movement towards the periphery, they have never been found in the outer part of the nucleus during this stage of oocyte growth. The fact that the occurrence of the nucleoli is restricted to the inner part of the nucleus is further evidence of the lack of any activity of the E-chromosomes in nucleoli production. Each of the nucleolus-forming bivalents, from which a nucleolus has moved off, starts producing new one. In this way, by the end of the first period of oocyte growth some 5 or 6 nucleoli can be present in the inner part of the nucleus.

During the initial period of nucleoli production the E-chromosomes still occur in the form of long, slightly twisted threads, dispersed at random in the outer part of the nucleus. Their behaviour and distribution in the nucleus do not indicate any relation to its inner part (Fig. 32). Towards the end of the first stage of the oocyte growth, however, this changes completely, when the E-chromosomes, while still showing no change in appearance, start to move towards the inner part of the nucleus (Fig. 33) and come eventually into contact with it. Then they twine themselves around it, frequently in such a way that at first only their middle parts are in contact with it, while their ends are outward bent (Fig. 34). Only after they have come quite close to the inner part of the nucleus, they adhere completely to its surface (Fig. 36).

While in the stage under discussion the inner part of the nucleus assumes a less regular shape, its size increases and its outline becomes less distinct. This possibly is the result of resumed production of perichromosomal substance which now surrounds the E-chromosomes accumulated on the surface of the inner part of the nucleus (Fig. 37).

As a result of these processes the inner part of the nucleus becomes a complex body, still containing in its interior the nucleoli-forming bivalents which now are surrounded by univalents. Both are immersed in a homogeneous substance. No traces of the border line of the original inner part of the nucleus containing only the bivalents are visible. Soon after entering the inner part of the nucleus the E-chromosomes, unlike the S-chromosomes, gradually become diffuse (Fig. 35). While only faintly stained chromatin granules can be seen of the E-chromosomes, the S-chromosomes are still deeply stained (Fig. 38).

Summing up, it seems possible to state that the process of gradual differentiation of the nucleus, commencing at the end of the first period of oocyte growth, leads to the incorporation of all E-chromosomes in the



Figs. 36—41. Fig. 36. E-chromosomes come into contact with the surface of the inner part of the nucleus. Fig. 37. The process of E-chromosomes inclusion into the inner part of the nucleus is associated with the appearance of a perichromosomal substance on their surface. Fig. 38. After incorporation into the inner part of the nucleus E-chromosomes lose their stainability. Fig. 39. Beginning of diffuse stage. Within the inner part of the nucleus, with the exception of nucleolus, weakly staining bivalents are still visible. Figs. 40 and 41. Diffuse stage. Period of intensive nucleoli production. Both the border of the inner part of the nucleus and the border between nucleus and cytoplasm are scarcely visible during this period. Figs. 36—38: hematoxylin. Figs. 39—41: gentian violet. ×2250

inner part of the nucleus. The process involves a characteristic reconstruction of the oocyte nucleus, resulting in its differentiation into two parts: an inner part which contains both the S- and the E-chromosomes, and an outer part which differs in structure from the former and contains no chromosomes.

b) The second period of the oocyte growth. The stage when in the inner part of the nucleus only S-chromosomes can be seen can be considered as start of the second period of the oocyte growth in ***Mikiola fagi.*** This stage precedes the period of very rapid growth of the nucleus and of the whole oocyte as well, which is accompanied by gradual resorption of the nutritive chamber.

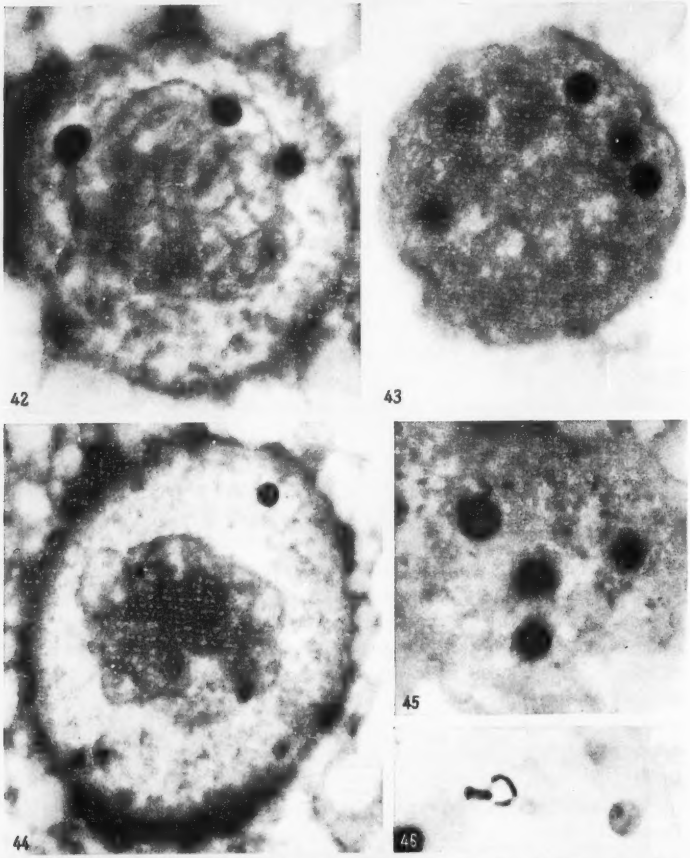
A phenomenon, frequently observed at the beginning of the second growth period, is the fusion of the nucleoli into one large nucleolus. It can usually be seen, that one or two bivalents are still in contact with the surface of this nucleolus (Fig. 39).

This stage, however, does not last long. Soon an enhancement of the nucleoli-forming activity of the still visible bivalents can be observed. This time the activity is manifested by a very rapid production of nucleoli of various sizes. As the number of nucleoli increases they gradually move from the inner part of the nucleus towards the nuclear membrane (Fig. 40). In this stage the boundary line between the nucleus and the adjacent layer of cytoplasm becomes very indistinct and is sometimes impossible to trace.

Soon after the start of the intense production of nucleoli the bivalents pass into a diffuse stage. Simultaneously, the inner part of the nucleus loses its stainability and its border lines become almost invisible. This is the only prophase stage, when the inner part of the nucleus loses its hitherto quite pronounced character (Fig. 41). Judging from the number of nucleoli the stage corresponds to a maximum of nucleoli-forming activity of chromosomes. It is, of course, not possible to detect whether it is the S-chromosomes only that continue to produce nucleoli in this stage or whether the E-chromosomes also take part in the process.

The nucleus, which has had an elliptical shape, corresponding to the flattened, crescent-shaped oocyte becomes spherical with the now beginning of rapid growth the oocyte. The nuclear border-line is distinct again, and on its surface appear small indentations (Fig. 43). Nucleoli are the only elements in the nucleus which at this stage can be stained with gentian-violet. All the rest of the nucleus when stained with this dye is yellowish-brown and shows no structure. However, after staining with iron hematoxylin the nucleus is not homogenous at this stage. In nuclei stained in such a way a nearly homogeneous peripheral part can be

distinguished, and against its background a spherical structure, having an irregular contour, is faintly visible in the centre. This structure seems to be composed of numerous, closely packed vesicles (Fig. 43).



Figs. 42—46. Figs. 42—44. Diffuse stage. Successive stages of the nucleus transformation in the second period of oocyte growth. The inner part of the nucleus is formed as if composed of intranuclear karyomeres. Fig. 45. Beginning of condensation of bivalents. Fig. 46. Early diakinesis. In this stage only bivalents are visible. One of them with three chiasmata can be seen. Fig. 42—44. Hematoxylin. Figs. 45—46. Gentian violet. ×2250

There is no doubt that there is a differentiation of the nucleus there, analogous to that occurring at earlier prophase. Thus, the peripheral part of the nucleus observed, is a zone of nuclear sap free from chromo-

somes, while the central structure is the changed inner part of the nucleus, which contains all the chromosomes. Observations of later prophase stages fully confirm this interpretation.

It seems rather unlikely that the differentiation of the nucleus observed is of secondary character. It should rather be assumed that during the initial period of the diffuse stage the inner part of the nucleus retains its individuality, although this is difficult to demonstrate.

The growth of the nucleus during the diffuse stage is accompanied by further changes in its structure. After staining with iron hematoxylin the changes are most pronounced in the inner part of the nucleus, which is now divided into numerous distinctly separated areas (Fig. 42). The latter, seem to be the previously observed vesicles, deformed as a result of their close packing. The inner part of the nucleus does not grow at the same rate as the whole nucleus, and this leads to a gradual broadening of the peripheral zone of the nuclear sap. Simultaneously, flocculent aggregations of a lightly stainable substance appear in the latter zone (Figs. 42 and 44). Towards the end of the diffuse stage each of the vesicles in the inner part of the nucleus becomes spherical, so that the whole inner part appears as an aggregate of intranuclear karyomeres (Fig. 44). During this and the preceding period of the diffuse stage this structure can be observed distinctly only when using iron hematoxylin. If stained with gentian violet the whole nucleus appears very faint, and both the border lines and the vesicles of the inner part of the nucleus are quite difficult to distinguish.

During these phases of growth of the oocyte nucleus the nutritive chamber is almost fully resorbed, the only trace left of it is a small aggregate of pycnotic nuclei, situated at one pole of the egg. Later, the rest of the nutritive chamber undergoes a complete degeneration.

While the oocyte is still growing and gradually attaining a cylindrical shape, the condensation of chromosomes in the inner part of the nucleus starts. At the beginning of this process some of the chromosomes, as can frequently be seen, appear in direct contact with the nucleoli (Fig. 45), which still are present in considerable numbers in both parts of the nucleus. Already at this stage it is clearly evident that only a few chromosomes become condensed. Shortly thereafter the four bivalents are visible in a form characteristic of diakinesis (Figs. 46 and 47). At this stage they are the only chromosomes observeable in the nucleus, but the appearance of the S-chromosomes is only slightly ahead of the condensation of the E-chromosomes. Soon, the latter can also be recognized in the inner part of the nucleus (Fig. 48). There can be no doubt, that in the diffuse stage the univalent E-chromosomes have undergone a substantial shortening, although they still appear as rather long threadlike elements located in the peripheral zone of the inner part of

the nucleus. Towards the end of the differentiation process of the E-chromosomes an increasing condensation of the bivalents takes place (Fig. 49).

Owing to scarcity of material containing oocytes in this prophase stage, it was not possible to establish whether condensation of the S- and the E-chromosomes proceeds, as it seems possible, in separate vesicles, or whether the boundaries between the vesicles disappear and their contents fuse to form a common medium for all chromosomes.

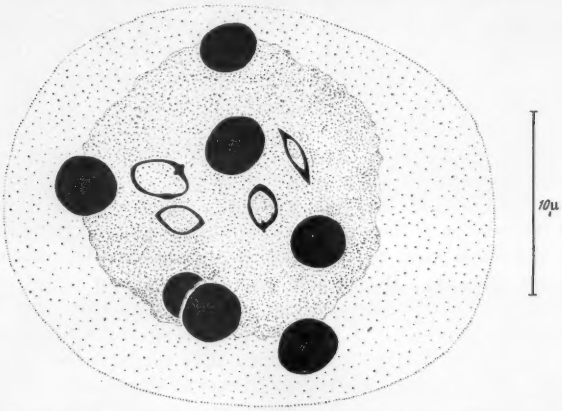
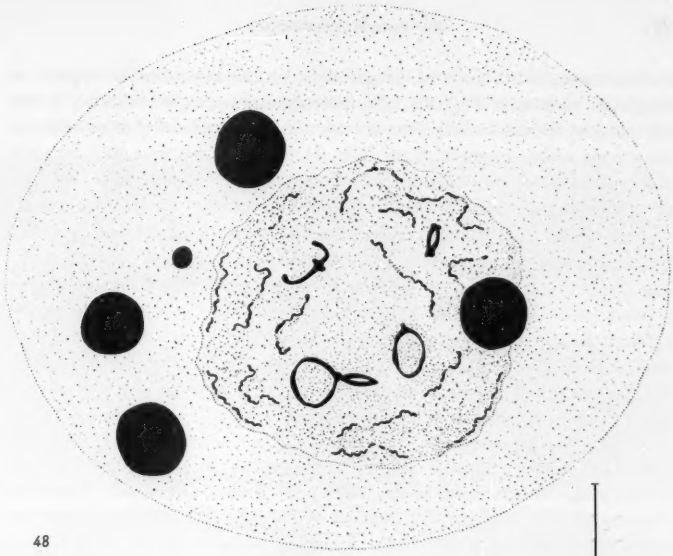


Fig. 47. Early diakinesis. Within the inner part of the nucleus four bivalents are visible. Gentian violet

The bivalents which appear at the end of the diffuse stage are mostly ring-shaped. This is consistent with the fact that in each of the diplotene bivalents two chiasmata are usually present. The presence of one or two nodes in ring-shaped bivalents (Figs. 47 and 49) indicates that the connection of the partners is, most likely, still of chiasmatic character and that the positions of the chiasmata are subterminal. Sometimes, however, there are no signs of such chiasmatic connections and the chiasmata seem to have undergone complete terminalization (Fig. 47). The appearance of a rod bivalent in Fig. 48 may be evidence of a complete terminalization of at least one chiasma and of the separation of the arms linked before by a chiasma.

As in the earlier prophase stages, also in this stage the bivalents undergo changes in advance of the E-chromosomes. The condensation of bivalents proceeds very rapidly and soon they become spherical, compact, readily stainable bodies, in which, however, the split between homologous chromosomes can still be seen (Figs 50 and 52). At the same time the



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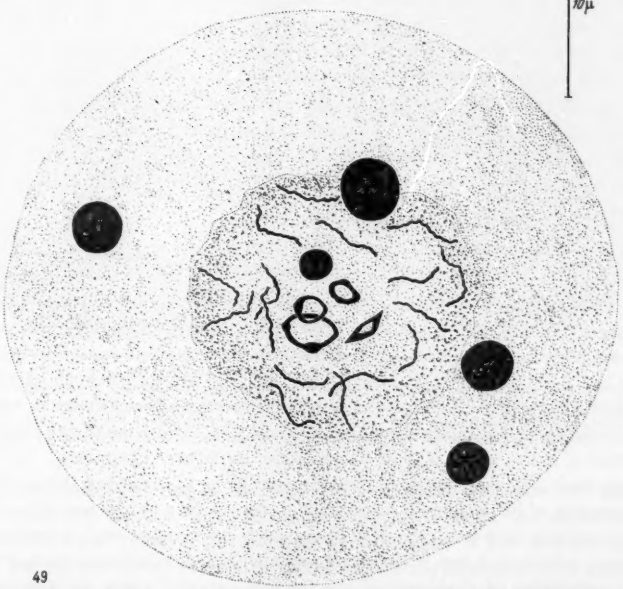


49

10μ

Figs. 48—49. Diakinesis. Condensation of E-chromosomes. While the bivalents show a high extent of condensation, the univalents still occur as relatively long and fine threads. All chromosomes assembled in the inner part of the nucleus. Gentian violet

E-chromosomes in spite of progressive condensation, still appear as elongated elements (Fig. 50). Frequently some deeply staining nodes can be seen located along the univalents, the internodes appearing as less deeply stained constrictions (Fig. 51). The E-chromosomes, in spite of a rather weak condensation, do not show chromatid splits, as they have been found by WHITE (1950) in *Phytophaga celtiphyllia*.

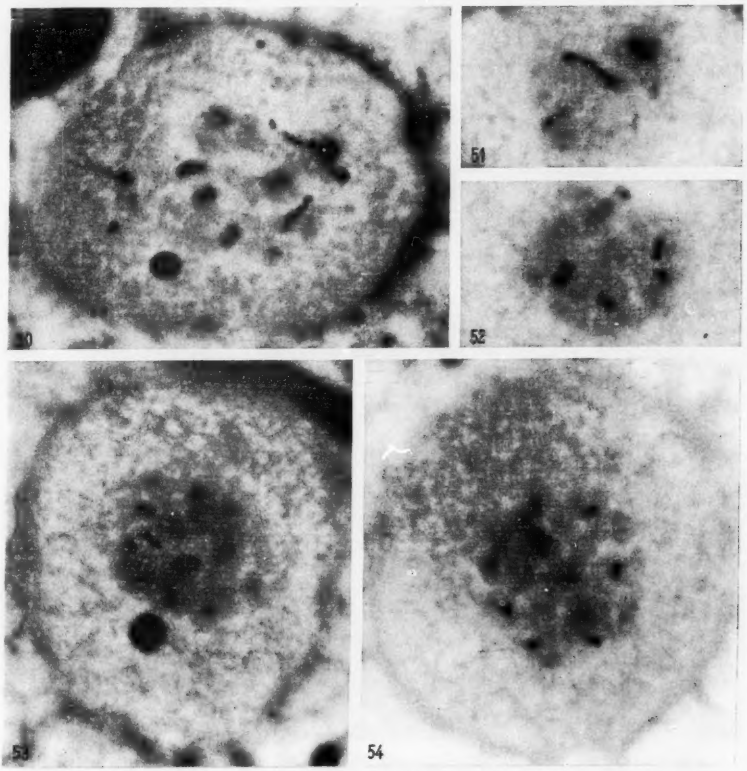
Condensation of the E-chromosomes soon transforms them into small rods uniformly stained over their entire length (Fig. 53). This is the stage, when the difference in length between various E-chromosomes is most pronounced. The longest one is nearly twice as long as the shortest ones. Exact classification as to different lengths was not possible, because they rarely were found lying in a focal plane but had mostly oblique positions. As the condensation of univalents proceeds, the inner part of the nucleus becomes spherical. Simultaneously its substance thickens, becomes opaque, is easier visible and distinctly contrasts with the wide, granular peripheral zone of nuclear sap (Fig. 55). In this stage the diameter of the inner part of the nucleus is $11.7 \pm 0.1\,\mu$. It seems as if it consists of a number of more or less delimited, chromosome-containing areas (Fig. 54). This might possibly be the result of fixation. On the other hand, the observed structure of the inner part of the nucleus might be an evidence of the differentiation of chromosomes from vesicles present during the diffuse stage, the inner part of the nucleus now consisting of the condensed karyolymph of the latter.

Towards the end of the second period of the oocyte growth the univalents and the bivalents are condensed nearly to the same degree, and only some of the univalents are still slightly elongated (Fig. 55). The nucleoli have completely disappeared both in the inner and the outer parts of the nucleus.

2. Prometaphase

Disappearance of the nuclear membrane and, similar to oogenesis of other *Cecidomyiidae* species (WHITE 1950), a considerable contraction of univalents are the first signs of transition from diakinesis to prometaphase. After the disappearance of the nuclear membrane both parts of the nucleus still retain their characteric properties although each of them has undergone considerable changes. Thus, the outer zone of the nucleus becomes more compact and its granular structure disappears. At the same time it becomes thinner, which seems to be the result of the gradual dispersion of its peripheral layers in the surrounding cytoplasm (Fig. 57). This process, however, is rather slow, since even at the end of prometaphase, which, judging from the frequency of its occurrence, lasts long, the remainder of that part of the nucleus can still easily be observed.

As a result of contraction, both the bivalents and the univalents are transformed into compact, spherical bodies of various sizes. Only the smallest of the chromosomes observed during this stage can be identified

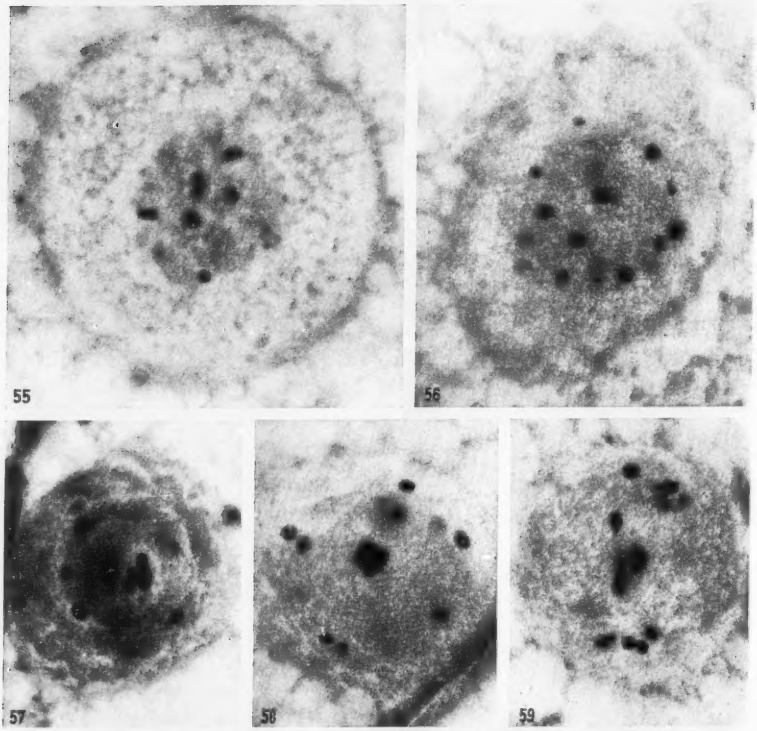


Figs. 50—54. Diakinesis. Fig. 50. Greatly condensed bivalents and univalents still relatively long. Fig. 51. One of the univalents in a stage analogous to that of Fig. 50. Fig. 52. The difference in degree of condensation of bivalents and univalents gradually decreases in later diakinesis stages. Some univalents and one bivalent visible in focal plane. Fig. 53. Differences in length of univalents. Fig. 54. Inner part of the nucleus consisting of a number of more or less distinct areas in which mostly a single chromosome lies. Gentian violet. ×2250

as univalents. The rest of the univalents, as far as their size and shape is concerned, do not differ from the bivalents. Thus, the distinction between both types of chromosome at this stage is not possible.

During the initial period of prometaphase any indications of spindle formation are lacking (Fig. 56). Only some time after the disappearance

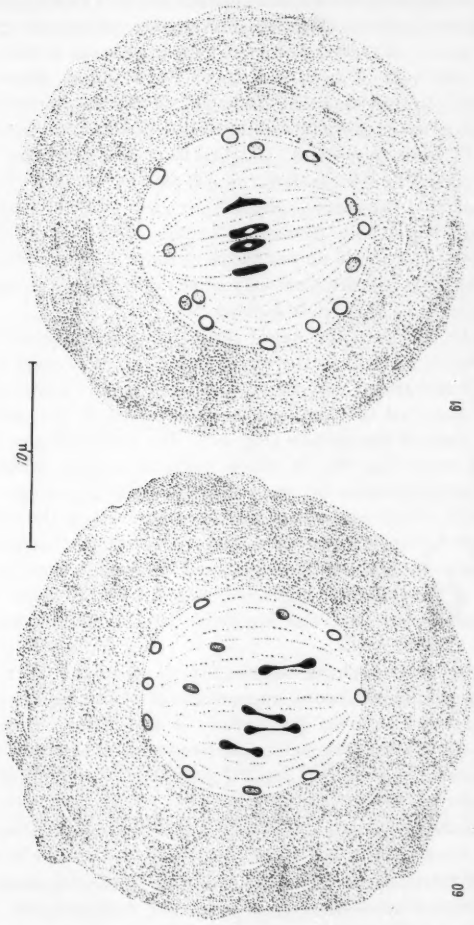
of the nuclear membrane, when the process of degradation of the outer part of the nucleus is already well advanced, the inner part of the nucleus undergoes a distinct contraction and becomes a regular sphere



Figs. 55—59. Fig. 55. Late diakinesis. The difference in degree of condensation between bivalents and univalents has almost completely disappeared. Fig. 56. Early prometaphase directly after disappearance of the nuclear membrane. The thickness of the outer part of the nucleus gradually decreases. Bivalents and univalents now appear as highly compact spherical bodies. Figs. 57—58. Inner part of nucleus transformed to a spindle. Bivalents with kinetochores oriented towards the poles now lie in the equatorial plane. Univalents uniformly distributed on spindle surface. Fig. 59. Prometaphase stretch of bivalents lying in the equatorial plane. Similarly as in Fig. 58 some of the univalents lie in very close neighbourhood of or even on the spindle pole. Figs. 55, 56, 58, and 59: gentian violet. Fig. 57: hematoxylin. ×2250

(Figs. 57 and 60). Thereafter the border line between the inner and outer parts of the nucleus becomes very distinct, while the diameter of the inner part of the nucleus is now only $10.5 \pm 0.1\,\mu$. Simulta-

neously with the contraction process a fibrillar structure appears in the karyolymph which occupies the inner part of the nucleus. This structure is the spindle. Size and shape of the developing spindle coincide



Figs. 60 and 61. Fig. 60. Early prometaphase. Prometaphase stretch of bivalents after transformation of the inner part of the nucleus into a spindle. Fig. 61. Appearance of prometaphase spindle with bivalents already in the equatorial plane. Both before and after congression of bivalents, the univalents are uniformly dispersed over the entire spindle surface. Hematoxylin

exactly with those of the inner part of the nucleus. It seems as if the latter were directly transformed into the spindle. It should also be kept in mind that during the formation of the spindle the inner part of the

nucleus still remains surrounded by a rather thick layer of the outer zone of the nucleus (Fig. 57). Simultaneously with the appearance of the spindle, separation of the kinetochores of the bivalents takes place; their further movement in the opposite directions leads to the prometaphase stretch of the bivalents (Fig. 60). The prometaphase movement of kinetochores is not accompanied, however, with any change in the length of the spindle which in *Mikiola fagi* keeps its spherical shape for a considerable part of prometaphase. Although during the prometaphase stretch the kinetochores gradually undergo quite distinct co-orientation, the directions of their movement, judging from the positions of the bivalents with regard to one another, are not always identical (Fig. 60). There is no doubt that the prometaphase stretch is a result of interaction between kinetochores and the developing spindle. Thus, since the kinetochores of different bivalents do not move in quite the same direction, it seems probable that a strict bipolarity of the developing, centriolless, intranuclear spindle in *M. fagi* is lacking.

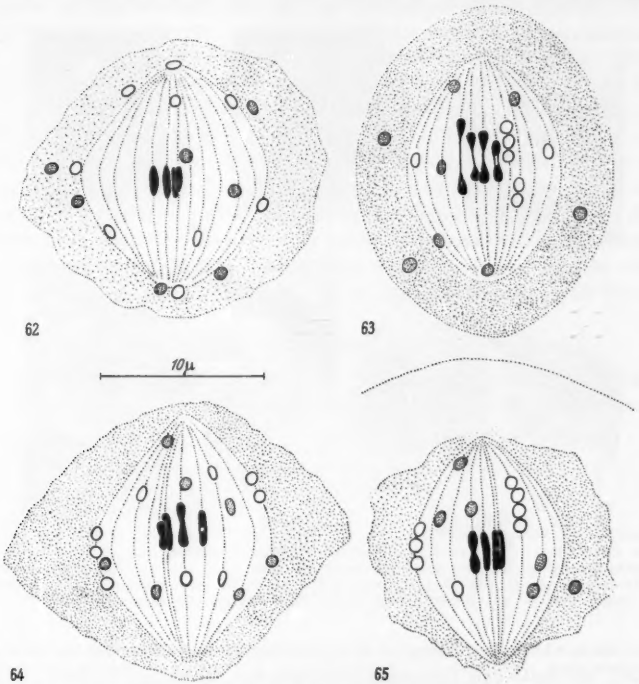
The bivalents, which in the initial period of prometaphase had no definite positions in relation to the equatorial plane, now start moving towards it. The movement may be accompanied by the contraction of the bivalents. Soon all the bivalents are assembled in the middle of the equatorial plane of the spindle (Fig. 57). The bivalents now appear mostly as long rods (Fig. 58), in which splits separating homologous chromosomes can sometimes be observed (Fig. 61). The shape of the bivalents located in the equatorial plane indicates that their kinetochores are rather far apart from each other. Although the shape of the spindle still shows no change, the movement of the bivalents towards the equatorial plane and their regular arrangement in it (Figs. 57, 58, and 61) are indications that the bipolarity of the spindle has been established.

Owing to the prometaphase contraction of the inner part of the nucleus and to the simultaneous appearance of the spindle, the univalents are displaced towards the surface of the latter. During the prometaphase stretch and the subsequent movement of the bivalents towards the equatorial plane the general pattern of distribution of the univalents shows no further change (Figs. 57, 58, 60, and 61). Both before and after the arrangement of the bivalents in the equatorial plane, the univalents remain quite uniformly distributed on the spindle surface with no indication of a concentration near the equatorial plane.

The univalents most frequently occupy the positions just at the border line between the spindle and the gradually disappearing outer part of the nucleus. In some cases, some of the univalents undoubtedly seem to be located outside the spindle, in the outer zone of the nucleus (Figs. 62 and 63). The occurrence of any fibres between the univalents

and the poles could not be demonstrated[1]. Not infrequently some of the univalents are quite close to, if not located at the spindle poles (Figs. 58—62).

The bipolarity of the spindle, which hitherto could only be deduced from the behaviour of the bivalents and from the increasing regularity

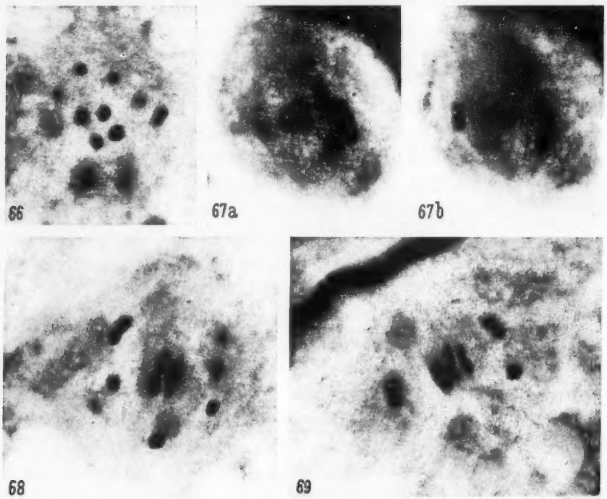


Figs. 62—65. Prometaphase. Fig. 62. Early prometaphase. Fig. 63. Prometaphase stretch of bivalents already in the equatorial plane. Some of the univalents lie in the outer part of the nucleus without any visible link with the spindle. Similarly as in Fig. 65. Linear associations of univalents are visible. Fig. 64. Late prometaphase. The number of univalents in the region of the poles has diminished. Fig. 65. Late prometaphase shortly before the formation of the metaphase plate. Outer part of nucleus already considerably reduced. Gentian violet

in the arrangement of its fibres, can now be observed by the gradual change of its shape. Thus, the spindle, initially still rather spherical (Figs. 59 and 62), becomes more and more elliptical and at the end of

[1] This, however, would be difficult to establish since in that stage the remaining outer zone of the nucleus is of fibrillar structure, somewhat similar to that of the spindle.

prometaphase has the shape of a typical anastral spindle, with more or less flattened ends (Figs. 65 and 67a). The spindle diameter decreases during the above transformation. Before metaphase plate formation it measures only $7.3 \pm 0.1\,\mu$. Although the length of the spindle is difficult to determine accurately, there can be no doubt that, as the diameter of the spindle decreases, it becomes more and more elongated.



Figs. 66—69. Late prometaphase. Fig. 66. Polar view of the group of bivalents within the spindle center. The number of univalents in the equatorial part of the spindle towards the end of prometaphase has increased. Figs. 67a, b, 68. Associations of linearly arranged univalents. Fig. 69. Stage immediately preceding the formation of the metaphase plate. Figs. 66, 68, and 69: gentian violet. Figs. 67a and 67b: hematoxylin × 2250

Both, at the beginning of spindle elongation and during the process itself bivalents can frequently be observed which are located in the equatorial plane and show a strong prometaphase stretch (Figs. 59 and 63). The number of stretched bivalents is different in various spindles. Towards the end of prometaphase all the bivalents show nearly the same degree of concentration and appear in the form of cigar-shaped bodies, some of which have slight constrictions in the middle (Figs. 65 and 69). Bivalents retaining at prometaphase or metaphase the length they had had at late prophase have been seen only rarely.

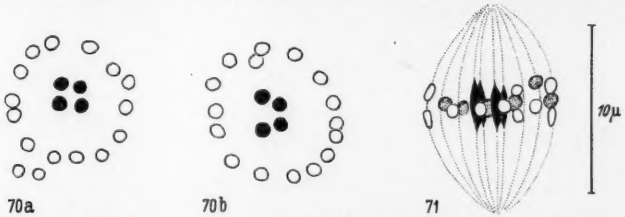
During the initial period of spindle elongation no visible changes in the distribution of the univalents can be seen (Fig. 62). However, as the process goes on the univalents move slowly towards the equatorial plane. This can be seen from an increasingly lower number of chromo-

somes near the poles and from their gradual congregation at the equator (Fig. 64). The univalents seem to move towards the equatorial plane from their initial positions on the surface of the spindle or just under its surface. In that way the univalents, after reaching the equator which is determined by the positions of the bivalents, always arrange themselves at the circumference of the metaphase plate (Fig. 66). The movement towards the equatorial periphery is often accomplished in a peculiar manner. Two, three or even four univalents follow each other in lines parallel to the spindle fibres (Figs. 63, 65, 67a and b, and 68).

As the formation of the metaphase plate proceeds, the univalents disappear more and more from the outer zone of the nucleus. There can be no doubt that their kinetochores gradually come into contact with the spindle, and then show a normal response to the forces which are acting in the spindle and which are responsible for the formation of the metaphase plate. It happens only rarely that some of the univalents present in the outer zone of the nucleus do not come into contact with the spindle and are not included among the chromosomes constituting the metaphase plate.

3. Metaphase

a) Metaphase plate. The decrease of the spindle diameter, initiated at early prometaphase, proceeds during the formation of the metaphase

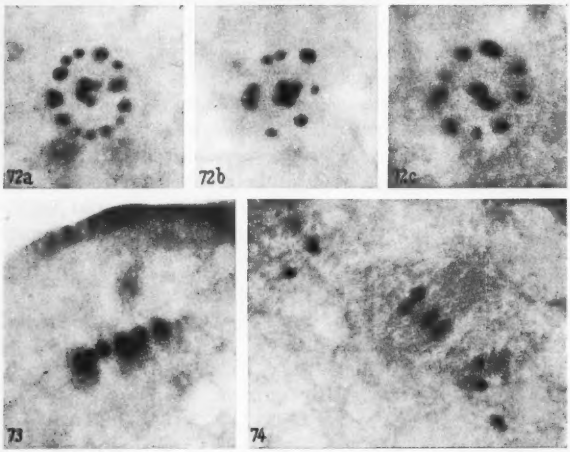


Figs. 70—71. Metaphase. Figs. 70a and b. Regular metaphase plates. In Fig. 70a two univalents outside the spindle. Fig. 71. Side view of metaphase plate. Some of the univalents lie above each other. Gentian violet

plate. At metaphase the spindle diameter is as small as $6.5 \pm 0{,}06\,\mu$. The remaining outer zone of the nucleus which could still be observed at the beginning of metaphase soon disappears completely.

In the first metaphase of *Mikiola fagi* (Figs. 70 and 72) the bivalents are always located inside the equatorial plate, the univalents on its periphery; however, the number of the latter, when viewed from above, varies within a wide range (Figs. 72a and b). In the initial period of the present work, this was the cause of misunderstanding and erroneous interpretations. This variation can be explained as follows: (1) the linear arrangement of the univalents, observed during late prometa-

phase persists in metaphase. Thus, not all the univalents are located exactly in one plane. Some of them are still exactly one above another, which frequently simulates the beginning of anaphase separation of homologous chromosomes (Fig. 71). (2) As mentioned before, some of the univalents, present during prometaphase in the outer part of the nucleus, do not participate in the formation of the metaphase plate and are located outside the spindle (Fig. 70a) at different heights with respect



Figs. 72—74. Metaphase and metaphase elimination of univalents. Fig. 72a—c. Appearance of metaphase plates before elimination of univalents. Figs. 73. Side view of regular metaphase plate. Fig. 74. Elimination of univalents from spindle. Gentian violet. ×2250

to the equatorial plane of the spindle. (3) During metaphase, neighbouring univalents stick together for some time (Fig. 72), which makes exact determination of the number of chromosomes in the plate very difficult.

Nevertheless, metaphase plates with nearly regular arrangements of their univalents (Figs. 70b and 72a) can often be found.

Both, during the prometaphase movement of the univalents towards the equatorial plane and after metaphase plate formation, the position of the spindle axis with respect to the surface of the egg remains variable. At metaphase the position of the spindle may be vertical, inclined or even tangential to the surface of the egg.

The metaphase plate which forms in the pupa of *M. fagi* long before the adult emerges from it is rather an unstable system and does not represent, while in the above forms, any starting point for the maturation divisions.

b) Elimination of the E-chromosomes and their behaviour outside the spindle. Neither signs of incipient anaphase movement of chromosomes nor any change in the appearance of both spindle and metaphase plate itself indicate the start of a new process which brings about drastic changes in the positions of the bivalents and univalents with respect to one another. This process starts with the sudden elimination of the univalents from the spindle. The elimination seems to proceed very rapidly. Most frequently all the univalents present in the metaphase plate are eliminated. The process of the elimination of univalents takes place in all eggs. When, as in some cases, some univalents remain in the spindle, then they are displaced from their initial positions (Figs. 76 and 86).

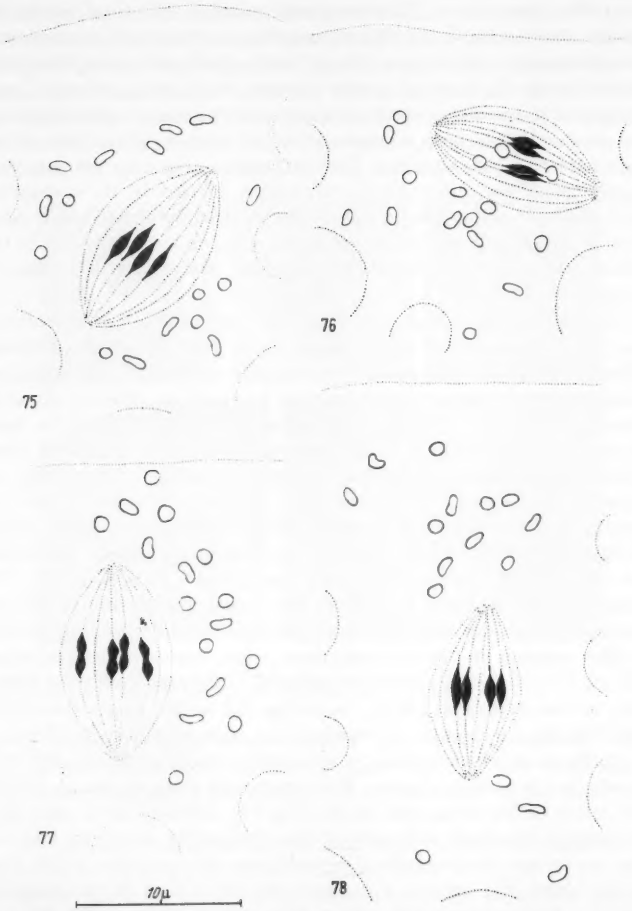
In slides which happen to show the very moment of elimination the univalents can be seen ejected from the spindle in different directions (Fig. 75), in extreme cases nearly exactly in the horizontal plane (Fig. 74). Ejected univalents can be found at different distances from their spindle. Usually, the distance is from a few to some 20 microns[1]. The contact of the univalents with the spindle is interrupted during their elimination into the cytoplasm. There were no connections observable between the eliminated univalents and the spindle, as is the case with spermatocytes of *Humbertiella indica* where HUGHES-SCHRADER (1948) did find a chromosomal fibre, connecting the expulsed X chromosome with one of the spindle poles. The behaviour of univalents in *Mikiola fagi* during the further stages also indicates the lack of any structural bond between them and the spindle.

The position of the bivalents during the elimination of univalents and the following stage remains unchanged. They still occupy the middle part of the equatorial plane. As during the earlier stages the spindle part with the bivalents is surrounded by a layer of continuous fibres.

In the stage which follows the elimination of univalents, the bivalents usually are of various shapes. Most frequently they appear as spindle-like, more or less elongated bodies (Fig. 91), although they may often be strongly stretched, and some of them frequently show only a thread-like connection between their homologous chromosomes which have moved apart (Fig. 90) for a distance similar to that in prometaphase stretch. Such a separation, although to a lesser degree (Fig. 74), was also observed in earlier stages.

Owing to variations in the spindle diameter during all stages of oogenesis, and to the fact that the author had only few slides showing the moment of elimination of the univalents, it is rather difficult to state if and to what extent the elimination

[1] Thus, my earlier statement (MATUSZEWSKI 1960) that univalents are often scattered in the cytoplasm within a radius of 30—40 μ, should be corrected. The srroneous measure of the distance is to be attributed to the fact that the observations were at first made on strongly squashed eggs.



Figs. 75—78. Fig. 75. Metaphase elimination of E-chromosomes from the spindle. Figs. 76—78. Various cases of dispersion of E-chromosomes eliminated from the spindle. Fig. 76. Not all E-chromosomes have been eliminated. A change is observed in the position of the non-eliminated E-chromosomes. Fig. 77. Shift of eliminated chromosomes to one side of the spindle. Fig. 78. The elimined univalents migrate towards the surface of the egg. Gentian violet

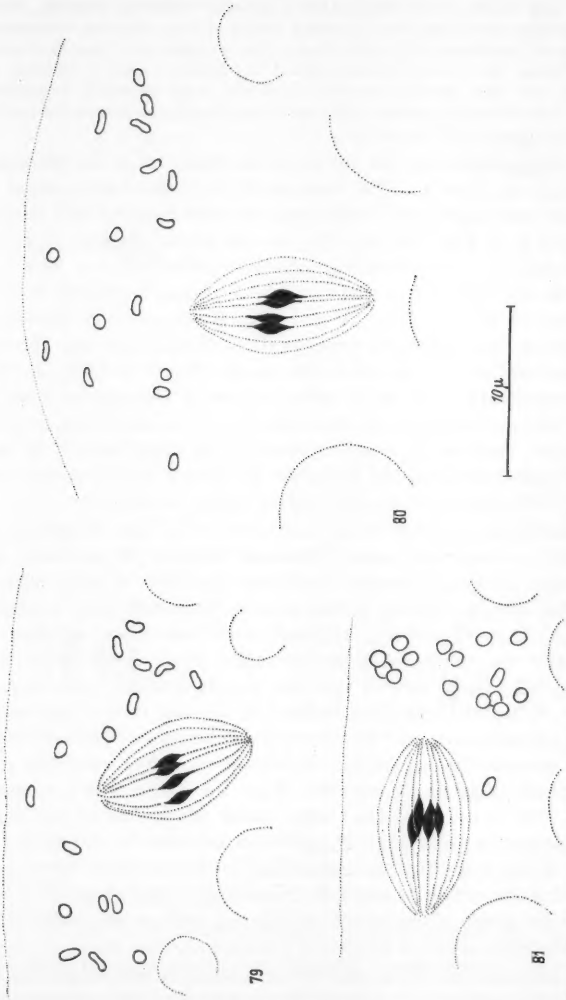
was accompanied by changes in the spindle itself. Nevertheless, any changes in the structure or shape of the spindle, both during the elimination and before it,

seem very improbable. It is believed that a further, substantial decrease in the spindle diameter occurs only after a certain period of time after the elimigation. The pattern of distribution of the univalents in the cytoplasm may be an indication of this. During the post-elimination period the spindle diameter decreases to $4.8 \pm 0.09\,\mu$ and then remains constant until the stage preceding maturation divisions. Nevertheless it remains still larger than that of the area in the spindle occupied by the group of bivalents.

Soon after elimination, the univalents are dispersed in the cytoplasm surrounding the spindle. The direction of their movement seems to depend on accidental forces, which result in their irregular and random distribution (e.g. Figs. 76 and 78). In the course of time, however, some regularities in the behaviour of the univalents can be observed. Thus, while still widely dispersed, the univalents slowly migrate towards the surface of the egg (Figs. 79 and 80). Moreover, in a number of cases prior to this migration towards the surface of the egg, the univalents moved towards one side of the spindle (Fig. 77 and 92). In spite of the considerable number of slides examined the author failed to establish factors responsible for the behaviour of the univalents in either of the cases. Anyhow, it seems certain that the orientation of the axis of the spindle containing the bivalents, which was found to vary considerably with respect to the egg surface, plays no role here.

No matter how the univalents have behaved in the cytoplasm, the end effect is always the same. Distances between the scattered univalents now gradually decrease, and soon they form a group situated close to the spindle. Usually at that time the univalents form a compact group (e.g. Figs. 93 and 95), although cases were observed where the group either was extended in the horizontal plane over a rather large area (Fig. 82) or was divided into two usually unequal parts (Figs. 81 and 83). When the spindle is inclined or vertical to the egg surface, the group of univalents most frequently lies either at the height of the pole directed towards the egg surface, or slightly above the equatorial plane of the spindle (Figs. 84, 93, and 94). When the spindle is in a tangential position, the univalents gather either under the surface of the egg, at one of the spindle poles (Figs. 81 and 96) or between the spindle and the surface of the egg (Fig. 85), depending on the distance between the spindle and the surface. Cases with tangentially oriented spindle located between the group of univalents and the egg surface, are rather exceptions (Fig. 87).

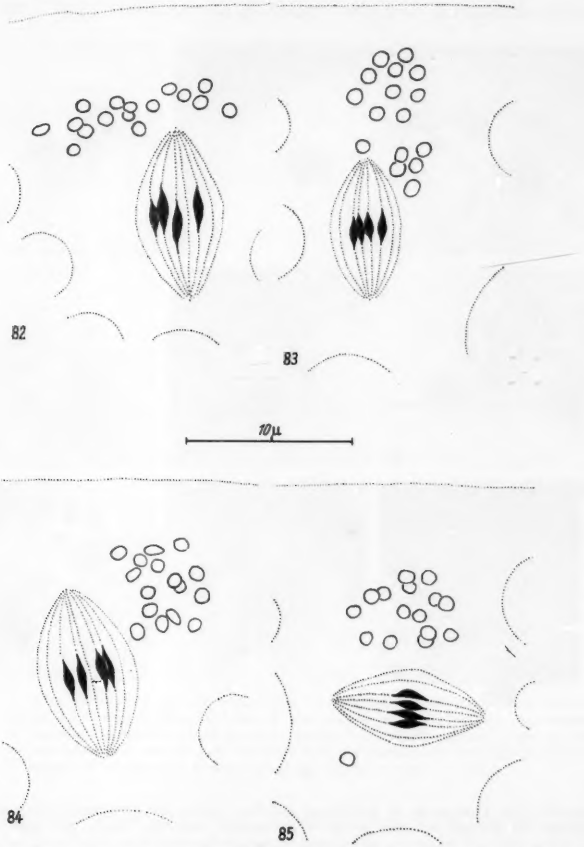
The arrangement of the univalents, united in this separate group, cannot be considered as random. It is often like the arrangement of chromosomes in a metaphase plate. In other words, most of the chromosomes of the group lie nearly in one plane (e.g. Fig. 95). Soon the similarity between the arrangement of the univalents in the group and



Figs. 79—81. Figs. 79—80. The still dispersed univalents assemble under the egg surface. Fig. 81. Univalents draw together to form one or two chromosome groups. Gentian violet

that of the chromosomes in the metaphase plate becomes still more pronounced, when fibrous, spindle-like structures appear in the cytoplasm near each of the groups of the univalents. The similarity between these

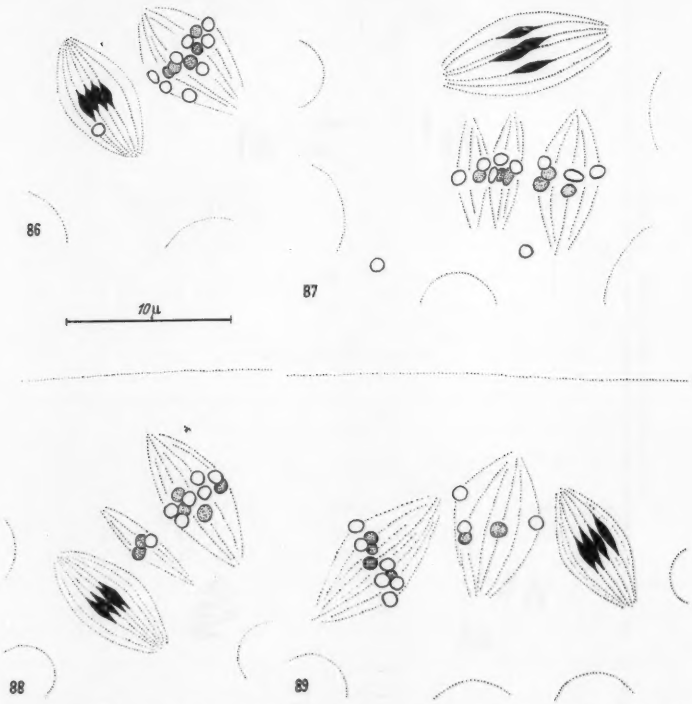
structures and ordinary mitotic spindles is evidenced not only by the presence of fibres in the former, but also by their spindle-like shapes. The only difference is that the arrangement of the fibers in them



Figs. 82—85. Formation of groups of eliminated univalents in the neighbourhood of the spindle. Gentian violet. See text for details

is less regular than in the bivalent-containing spindle. Thus, we are in a position to observe the formation of additional mitotic figures by the univalents eliminated into the cytoplasm; this process is not preceded by the formation of interkinetic nuclei. In accordance with

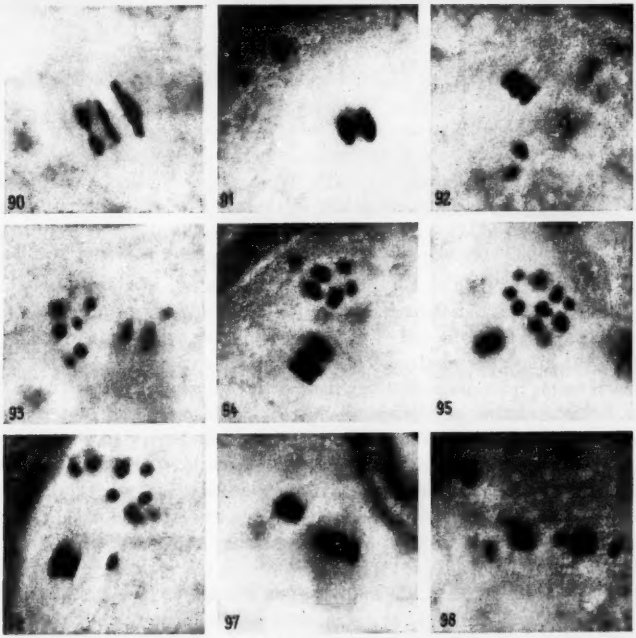
what has been said about the behaviour of univalents after their elmination from the spindle, the presence of one additional mitotic figure in the egg is the most frequent case (Figs. 86 and 97). The presence of two



Figs. 86—89. Formation of additional spindles containing eliminated univalents. Such spindles are formed for each group of univalents. Gentian violet. See text for details

or three additional mitotic figures (Figs. 87—89, and 98) can be observed only rarely. The univalents are, as a rule, situated in the equatorial zones of spindle-like structures, their arrangement is, however, usually irregular. In some cases, apart from additional mitotic figures, some dispersed univalents can be seen in the cytoplasm of the egg (Fig. 87). The diameters of the additional spindle-like structures, unlike that of

the spindle containing the bivalents, are equal to the diameters of the chromosome groups, which they contain. The axes of additional mitotic figures are oriented mostly parallel to the axis of the bivalent-containing spindle (Figs. 86, 88, and 97) or, in some cases, they form angles with it (Fig. 89). Only when a tangentially oriented bivalent-containing



Figs. 90—98. Fig. 90. Spindle with bivalents after elimination of E-chromosomes. Figs. 91 and 92. Spindle and eliminated univalents in the course of migration to egg surface. Figs. 93—96. Spindle and neighbouring group of eliminated E-chromosomes. Figs. 97—98. Spindle with bivalents and one (Fig. 97) or two (Fig. 98) additional spindles formed by groups of eliminated E-chromosomes. Gentian violet. × 2250

spindle is located between a group of univalents and the surface of the egg, the axis of the additional mitotic figure is vertical to the spindle axis (Fig. 87).

c) Return of the E-chromosomes on the spindle. The stage when additional mitotic figures occur lasts for a rather long time, until the adults start emerging from the puparia. Then the configuration of chromosomes in the egg undergoes a new, striking change. Eggs, shortly before they are laid, and newly laid eggs as well, no longer contain additional

mitotic figures. Presently, only one spindle can be observed in the egg which includes again the bivalents and the univalents.

The period from the moment when the first sign of univalent elimination from the spindle is observable to the moment when imagos emerge, is relatively long and lasts in *Mikiola fagi*, under the conditions previously described, at least several weeks. Within the above period of time the number of eggs in the stage of univalent elimination and of formation of additional mitotic figures gradually increases, while the frequency of metaphase plates containing univalents decreases both intra- and interindividually. Towards the end of this period univalent-containing metaphase plates are no longer present. On the other hand, after adults have emerged, the stage characterized by the presence of additional mitotic figures becomes increasingly rarer, whereas the number of eggs with only one spindle increases. The above observations, as well as the appearance and diameter of the spindle and the distribution of the univalents on the spindle, exclude the possibility that there are eggs in *M. fagi* in which the process of elimination of univalents from the metaphase plate has been omitted, which would infer that if only one spindle is present in the egg after the imagos have emerged it is an egg in which the above elimination has not taken place at all. Thus, the conclusion seems reasonable that the reorganization which takes place in the egg before maturation divisions, but after the emerging of imagines, consists in the disappearance of the additional spindle and in the inclusion of the univalents in the bivalent-containing spindle. The above processes must proceed at a considerable speed, for they have never been observed.

The only spindle now present in the egg is as a rule in a position vertical to the egg surface. It follows from this, that at the time, when the univalents join the spindle containing the bivalents, the spindle often undergoes rotation, dependent upon the initial position of its axis.

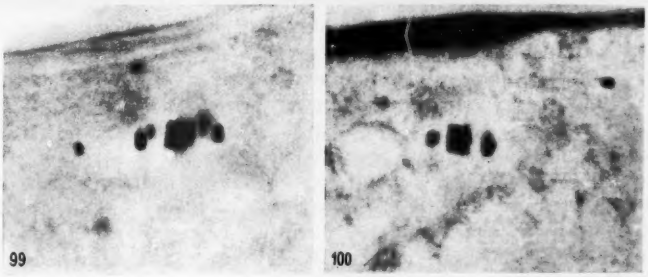
The arrangement and the appearance of the bivalents in the spindle during all these transformations remain unchanged. They still occupy the middle of the equatorial plane of the spindle. The diameter of the spindle, after the univalents have entered it, increases to $5.5 \pm 0{,}08\,\mu$.

The fluctuations in the spindle diameter during oogenesis in *M. fagi* are difficult to explain. It is, however, possible that this last increase is a result of the fusion of the spindles.

After joining the spindle the univalents are distributed only on the surface of the spindle. This arrangement is similar to that after the formation of metaphase plate at the end of prometaphase, but this time it is considerably less regular.

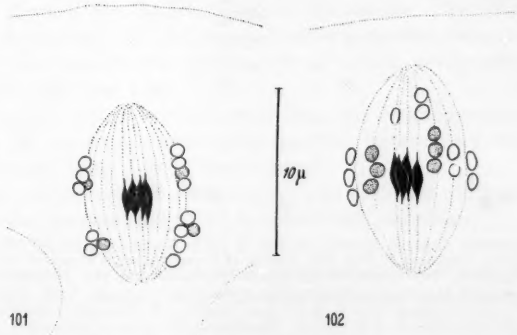
Only a few of the univalents remain isolated after their re-entry into the spindle; the rest of them form several groups, each consisting of 2—4 elements (Figs. 101 and 102). The univalents in a given group are usually arranged in chains parallel to the spindle axis. This

arrangement and the loose contact between the univalents in the chain are very much like the configurations of the univalents during the process of the metaphase plate formation at prometaphase. The presence



Figs. 99—100. Metaphase shortly before first maturation division. The secondary character of the relatively regular metaphase plates is indicated by the presence of univalents dispersed in the cytoplasm. Gentian violet. ×2250

of the groups of univalents on the surface of the spindle and their distribution at various heights with respect to the equatorial plane account for a very characteristic appearance of the spindle at this stage.



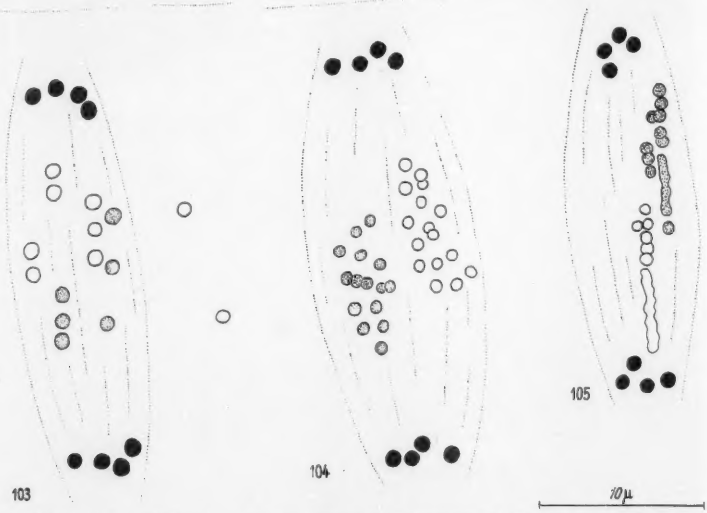
Figs. 101—102. Metaphase preceding first maturation division. Univalents joint to the spindle after disappearance of additional mitotic figures. Groups of linearly arranged univalents lie at various levels on the spindle surface. Gentian violet

The aggregates of univalents as well as the rare single ones occupy positions at the circumference of the equatorial plate of the spindle (Figs. 99 and 100) thus forming a secondary more or less regular metaphase plate. In these and in other cases the secondary character of such a plate can be inferred from the presence of single univalents dispersed in the cytoplasm and frequently situated far from the spindle (Figs. 99 and 100). The first maturation division, however, seems mostly

not to be preceded by the formation of such a fairly regular metaphase plate. On the contrary, patterns of maturation divisions seem to confirm the assumption that the distribution of the groups of univalents at various heights with respect to the equatorial plane before the division is normally only slightly changed.

4. Maturation divisions

Under the conditions described on p. 743 which most probably occur in numerous cases also in nature, the two maturation divisions



Figs. 103—105. Anaphase I. Fig. 103. Groups of E-chromosomes dispersed on interzonal part of the spindle. Two univalents still free in cytoplasm. Fig. 104. Anaphase separation of two groups of daughter E-chromosomes towards the opposite poles of the spindle. Fig. 105. Great elongation of each of the two daughter E-chromosome groups. Gentian violet

take place in laid but unfertilized eggs. The late anaphase stage of the first maturation division was the first one the author was able to observe in a number of eggs. But, as the patterns of this stage were very clear, both the behaviour of chromosomes and changes in the shape of the spindle at early anaphase could easily be reconstructed from them.

a) The S-chromosomes. At late anaphase I (Figs. 103—105) four spherical, compact, deply stained bodies are always observed at each of the spindle poles. Although their appearance in this stage does not

indicate them to be composed of two halves, there is no doubt that they are the four dyads resulting from the anaphase separation of the S-chromosomes. Judging from their appearance and nearly regular arrangement at late anaphase, one can infer that at early anaphase their separation proceeds in an orthodox manner. Since the dyads are located close to the spindle poles and the spindle is rather long, the anaphase movement of the S-chromosomes can be assumed to be accompanied by shortening of the chromosomal fibres and by elongation of the spindle itself (comp. Figs. 101—105).

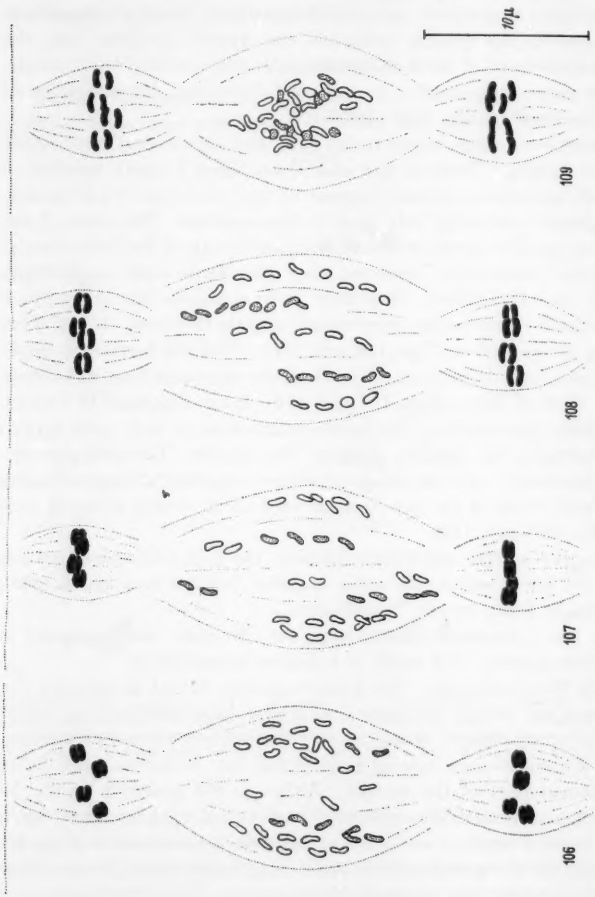
An interphase stage between the first and the second maturation division is lacking. Towards the end of anaphase I, small spindles of the second maturation division appear at the two ends of the spindle, each of them containing only four S-chromosomes. The axes of the spindles are usually continuations of the spindle axis of the first maturation division. Sometimes, however, they may form slight angles with the latter, and in addition, they may lie in not quite the same plane. The spherical S-chromosomes now become slightly flattened, each of them consisting of two halves (Figs. 106 and 107). After the formation of the second maturation division spindles, the S-chromosomes form metaphase plates in each of them (Figs. 107 and 110). Soon anaphase II follows, during which chromatids of the S-chromosomes move in a quite typical manner towards the opposite poles of the spindle. The strongly condensed chromatids take the shape of either somewhat elongated bodies slightly bent towards the poles, or of very short, nearly straight rods (Figs. 108, 109, and 111).

During the second maturation division, the S-chromosomes, present both in the inner and in the outer spindles, behave very much alike, their division being closely synchronized.

Thus, the S-chromosomes undergo two divisions which proceed in an orthodox manner, and result in a numerical reduction.

b) The E-chromosomes. The E-chromosomes do not accompany the S-chromosomes during the normal anaphase movement of the latter. At the end of anaphase I, when the spindle attains its maximum length, and the S-chromosomes approach its poles, the E-chromosomes lie in the interzonal part of the spindle. Although the positions of the E-chromosomes respective of one another have changed, this seems to be in the first place a result of anaphase elongation of the spindle. They are scattered in the elongated spindles mostly in groups of two, three or four (Figs. 103 and 110) not as single chromosomes. The groups are most probably those formed by the E-chromosomes just before the first maturation division (Figs. 101 and 102). Owing to the spindle elongation, distances between the univalent E-chromosomes in separate groups seem to increase only insignificantly. The E-chromosomes

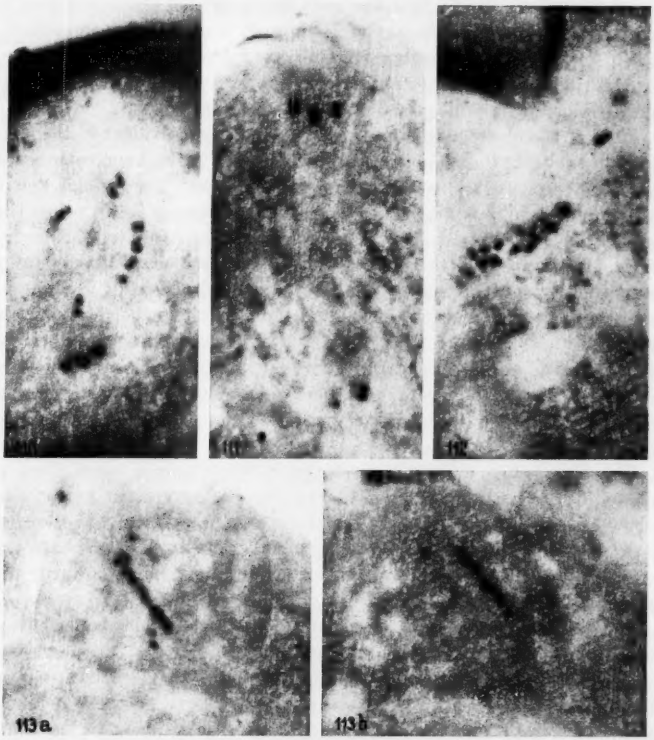
still show a high degree of condensation (Fig. 103) during anaphase I and probably, therefore, the split between the chromatids cannot yet be observed. In some cases some univalents can also be seen outside



Figs. 106—109. Figs. 106—107. Metaphase II of S-chromosomes. Fig. 106. Equational splitting of E-chromosomes. Fig. 107. Initial stage of anaphase movement of daughter E-chromosomes. Figs. 108—109. Anaphase II of S-chromosomes. Fig. 108. Owing to anaphase movement of daughter E-chromosomes, long rows of linearly arranged chromosomes can be seen on the spindle surface. Fig. 109. Two anaphase groups of daughter E-chromosomes. Gentian violet

the spindle (Fig. 103). These are undoubtedly E-chromosomes which had been eliminated from the primary metaphase plate and did, for unknown reasons, no reenter the bivalent-containing spindle.

During the period of time between late anaphase I and the formation of the small spindles of the second maturation division containing only the S-chromosomes, the spindle of the first maturation division



Figs. 110—113. Maturation divisions. Fig. 110. Metaphase II. Inner spindle of second maturation division containing S-chromosomes. On the surface of the first meiotic spindle lie rows of equationally splitting E-chromosomes. Fig. 111. Anaphase II. Outer spindle of second maturation division with dividing S-chromosomes. In the lower part of the photograph one of the dividing S-chromosomes is visible in the inner spindle of the second maturation division. Fig. 112. One of the anaphase groups of daughter E-chromosomes. Figs. 113a and b. Two very elongated anaphase groups of daughter E-chromosomes lying at various levels. Figs. 110—112. Gentian violet. Fig. 113. Feulgen. ×2250

undergoes considerable changes. While it becomes only a little shorter, its diameter increases significantly. As a result it becomes a barrel-like body with its ends most frequently remaining in contact with the inner ends of the two small second maturation spindles.

The fibrillar structure of the body is only very slightly manifest. But the same can be said of fibres in the spindles of the second maturation division. No matter whether the fibrillar structure of the transformed spindle of the first maturation division is easy to distinguish or not, there is no doubt that the body has a distinct shape and that it differs from the surrounding cytoplasm, and, what is more important, that it will serve for a regular distribution of dividing E-chromosomes. For this reason in the further description of the behaviour of E-chromosomes the body is still given the name of spindle.

Most of the observations on the division of the univalent E-chromosomes have shown that it occurs at the time when the S-chromosomes are in the metaphase of the second maturation division. The division starts with simultaneous splitting of each the E-chromosomes. As a result the number of the E-chromosomes increases to 32. The daughter E-chromosomes, thus formed, like the S-chromosomes at anaphase II, are mostly short, slightly curved rods. The kinetochores of the dividing E-chromosomes are now not oriented towards the poles of the spindle. This does not mean, however, that the positions of the daughter E-chromosomes with respect to the poles are quite irregular. On the contrary, in all of the cases analyzed the positions of most of the daughter E-chromosomes are such, that their long axes are parallel to the axis of the spindle (Fig. 106—108).

The time when the division of the E-chromosomes takes place is not definitely fixed. In some cases the division may occur as early as during anaphase I, the lag between the above process and the division of the S-chromosomes being so insignificant that at late anaphase the separation of the E-chromosomes into two groups moving towards the spindle poles is already well advanced (Figs. 104 and 105). In all such cases the S-chromosomes appear as spherical, compact bodies, thus distinctly differing from the E-chromosomes which in typical cases divide only when the S-chromosomes are at metaphase II. This speed-up of the division of E-chromosomes was found to have no effect on their further behaviour, and the end result of oogenesis seems to be the same as in typical cases.

After division of kinetochores the daughter E-chromosomes separate slightly from each other in a direction transverse to the spindle axis (Figs. 106 and 107). Only now the anaphase movement of the daughter E-chromosomes seems to start. The movement consists in a slow migration of the chromosomes towards the two poles of the spindle. Their kinetochores remain, however, still unoriented. As a result of this anomalous anaphase movement each of the chromosomes is most frequently oriented towards the pole with one of its ends and not with the kinetochore. It was not possible to determine how the E-chromo-

somes are connected with the spindle, owing to their small size and to difficulties in detecting the fibres of the spindle.

The small distances between the daughter E-chromosomes after their separation in the direction transverse to the spindle axis before the anaphase and their anomalous positions in the spindle soon, after their anaphase movement has started, cause them to arrange one above another, nearly in one line somewhat in an end-to-end grouping. In this way shorter or longer chains of daughter E-chromosomes are formed, depending on the number of chromosomes in separate groups dispersed in various planes in the interzonal part of the spindle at anaphase I (Figs. 107 and 108).

Not all daughter E-chromosomes start their anaphase movement simultaneously. While some of them show the end-to-end orientation, others remain in their positions, maintaining their nearly parallel arrangement (Fig. 107).

In accordance with the arrangement of the univalents in the secondary metaphase plate, the occurrence of chains of the daughter E-chromosomes in the initial period of anaphase is limited to the superficial layer of the spindle. That is why curved chains of the E chromosomes can frequently be observed, the curvatures of which correspond strictly to that of the spindle (e.g. Fig. 110).

In typical cases, at the time when the S-chromosomes are in anaphase of second maturation division, the arrangement of the E-chromosomes undergoes a change. The change consists, both in the above cases and when the E-chromosomes divide at anaphase of the first maturation division, in a segregation of the chromosomes into two groups. The details of this process are not yet known. Appearance and positions of the groups in the spindle with respect to one another are not like the configuration characteristic for a normal anaphase separation of daughter chromosomes (Figs. 104 and 109). The chromosomes of each of the groups, extended along the spindle, are scattered over a relatively large area (Fig. 112) and the groups lie in different planes. There is no doubt that the two groups of E-chromosomes move towards the opposite poles of the spindle (Fig. 104). In later anaphase stages the groups, as they approach the poles, become considerably elongated, and many of their chromosomes arrange themselves in chains (Figs. 105 and 113a and b).

Neither intermediate stages of anaphase segregation of the E-chromosome groups nor any visible differences in the appearance of the chromosomes have been seen. Therefore, it was not possible to determine whether it is daughter chromosomes that undergo segregation or whether the segregation is at random. In a few cases, however, it was possible to count the chromosomes in both, or at least in one anaphase group.

As expected, each of the groups contained 15—17 E-chromosomes. This seems to be an indirect proof of a regular, although not typical, segregation of the daughter chromosomes to the opposite poles.

The course of the maturation divisions in oocytes of *Mikiola fagi* seems to be quite clear: while the bivalent S-chromosomes undergo two divisions and thus are reduced in number, the E-chromosomes, owing to their more or less retarded mitotic cycle, undergo but one equational division which is largely independent of the S-chromosomes. Due to the unique role of the interzonal part of the first maturation division spindle, the daughter E-chromosomes move towards opposite poles.

Although the reconstruction of the egg nucleus was not observed, the conclusion seems reasonable, that the egg nucleus is formed by the union of one haploid set of the S-chromosomes with one group of E-chromosomes.

5. Prophase of the first cleavage division

It was not possible to obtain larvae or embryos of *Mikiola fagi* in large numbers in the laboratory. Consequently only fragmentary results have

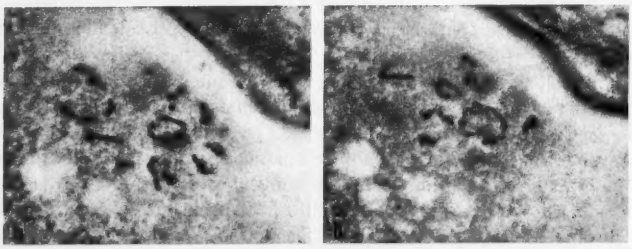


Fig. 114a and b. Prophase of the first cleavage division. Two pictures of the same nucleus at various levels. Probably this is an egg nucleus. Gentian violet. ×1250

been obtained. Several times eggs were observed which, while showing no signs of degeneration, each contained two nuclei with condensing chromosomes, the appearance of which indicated, undoubtedly, that they had already undergone maturation divisions. The nucleus closer to the egg surface was certainly a compound polar body, while the other, shifted towards the middle of the cell, was the egg nucleus ready for the first cleavage division.

In all cases observed, both the nucleus of the egg and the polar body were in the same division stage, viz. in late prophase or prometaphase. To distinguish the S-chromosomes from the E-chromosomes was impossible in these stages. All of them appear as long, thin, loosely spiralized threads (Fig. 114a and b), and thus are very much like prophase chromosomes during first cleavage divisions in *Monarthropalpus buxi* (WHITE

1950) and in *Miastor sp.* (NICKLAS 1959). An exact count of the number of chromosomes in this stage is very difficult to make, the nuclei of some eggs were found to contain approximately 22—24 chromosomes.

The degree of the chromosome condensation indicates that as in *Miastor metraloas* (KAHLE 1908) and in *Oligarces paradoxus* (REITBERGER 1940, HAUSCHTECK 1959) the polar body in *M. fagi* is most probably capable of normal division.

Finally, the fact should be emphasized that like the maturation divisions the prophase of the first cleavage division in *M. vagi* eggs has been found to occur in unfertilized eggs.

D. Discussion

I. Characterization of the S- and E-chromosomes and some general problems of mitosis

The differentiation of the karyotype into a set of S-chromosomes and a set of E-chromosomes, characteristic of all *Cecidomyiidae* is manifest in all stages of the chromosome cycle of the members of the subfamily *Cecidomyiinae*. In no stage of the cycle does the differentiation appear more distinctly than in the oogenesis in sexual females of *Mikiola fagi*.

The especially aberrant peculiarities of oogenesis in *M. fagi* can be attributed to the behaviour of the E-chromosomes. Since the problem of the origin of E-chromosomes has not been solved as yet, an analysis of their behaviour in different species in various stages of the chromosome cycle seems to be helpful in ascertaining their relation to S-chromosomes. Moreover, analysis of the behaviour of the E- and S-chromosomes might, despite the anomalous character of the *M. fagi* oogenesis, or perhaps due to it, shed some light on some problems of mitosis.

1. Prophase

The occurrence of the E-chromosomes as univalents during oogenesis was first observed by WHITE (1950) in a number of species belonging to the subfamily *Cecidomyiinae*. The course of the prophase stage of oogenesis in *Mikiola fagi* indicates the occurance of E-chromosomes also as univalents from the time of their appearance in the first period of oocyte growth. They were not found to undergo any form of pairing during the prophase stages.

Both at prophase and in later stages of oogenesis in *M. fagi* all of the E-chromosomes exhibit some common features differing from those of the S-chromosomes.

In time of condensation the E-chromosomes are behind the S-chromosomes during the whole prophase period. Only towards the end of the prophase the difference in degree of condensation between the E- and S-chromosomes appears greatly diminished. Doubtless, the high condensation rate of the S-chromosomes is closely related to their heteropycnosis in oogonia.

The S-chromosomes also substantially surpass the E-chromosomes with respect to the production of the perichromosomal substance which is a main component of the inner part of the oocyte nucleus. The latter start taking part in the process only towards the end of the first period of the oocyte growth.

E-chromosomes were not found to participate in the production of nucleoli. It may, however, be possible that their participation in this process is also delayed as compared with the S-chromosomes and that it does not begin before the onset of the diffuse stage.

2. Prometaphase

Recent studies (SHARMAN and BARBER 1952; SMITH 1952, 1953; MELANDER and KNUDSEN 1953; DIETZ 1954, 1956, 1958; STAIGER 1954; BAYREUTHER 1956; JOHN 1957a and b; JOHN and LEWIS 1957; WOLF 1960) have confirmed earlier suggestions (HUGHES-SCHRADER 1947; SCHRADER 1947, 1953; ÖSTERGREN 1951) that the prometaphase stretch in meiosis is a common phenomenon. It has been found to occur in members of such distantly related groups as *Insecta*, *Crustacea*, *Mollusca*, *Nematoda* and *Mammalia*. Moreover, it was often found here as distinct as in *Mantoidea*, *Phasmidea* and *Blattidea* of the *Orthoptera*. The asynchrony in the stretch of bivalents and in their subsequent shortening and congression first observed by HUGHES-SCHRADER (1943, 1947) has also been found to be a common phenomenon, which is convincing evidence of the autonomy of chromosomes at the prometaphase.

Although the degree of the prometaphase stretch in different species may vary considerably, and although its maximum may occur either at the beginning or at the end of prometaphase, the prometaphase stretch of the bivalent seems always to occur only after its kinetochores have come into contact with the developing spindle. The stretch of a bivalent is the result of the action of two forces applied to its kinetochores, the forces being directed towards the opposite spindle poles. DIETZ's (1956) observations on the movements of the chromosomes during the prometaphase of meiosis in *Tipula lateralis* seem to confirm the above suggestion. DIETZ found both the movement of the bivalents in the spindle and their prometaphase stretch to be results of the action of the same forces. A confirmation of the hypothesis that the beginning of the prometaphase movement of kinetochores depends on the inter-

action between the latter and the developing spindle is provided by observations on the mitosis in the endosperm of various plant species (BAJER 1954, 1958; BAJER and MOLÉ-BAJER 1956). The lack of the metaphase stretch in cells treated with inhibitors of spindle formation (JOHN and LEWIS 1957) is another confirmation of the above hypothesis.

From his observations on the movements and changes in shape of the bivalents and multivalents at prometaphase, DIETZ (1956, 1958) infers that pulling forces acting during prometaphase on the kinetochores in the direction of the poles gradually diminish, to remain at metaphase at a minimum, which may be equal to, or only slightly higher than zero. According to his hypothesis, changes in the strength of pulling forces during the prometaphase and metaphase are brought about by changes in lengths of chromosomal fibres. Thus, at the beginning of prometaphase substantial pulling forces, directed towards the poles, are acting on the kinetochores. The forces are the result of the contraction of developing chromosomal fibres. Unless the resistance of the chromosomes to the deforming forces is very high, the chromosomal fibres due to their contraction become shorter and a bivalent or a multivalent is stretched. Otherwise, the fibre does not shorten, its contraction is "isometric" and the only sign of the forces acting at prometaphase on the unstretched bivalent is its slight oscillation between the poles. The movement is brought about by slight, local changes in the contraction of the two chromosomal fibres. The shortening of chromosomal fibres, as the result of their contraction is gradually compensated, at prometaphase, by the growth of the fibres which is due to the incorporation of new amounts of the substance, they consist of. The elongation of chromosomal fibres is accompanied by a decrease in the strength of the pulling force exerted by the fibres on kinetochores. The decrease results in the disappearance of the prometaphase stretch of the bivalents. The process continues until metaphase. At metaphase, chromosomal fibres attain a maximum length which is only slightly less than half-spindle length. They keep the chromosomes in the equatorial plane until the anaphase starts and exert only an insignificant, if any, pulling force on the chromosomes.

DIETZ (1958) explains the changes in the shapes of bivalents at prometaphase by assuming changes in the strength of external forces, acting on the chromosomes through their kinetochores. Thus, he rejects the possibility that the shortening of the bivalents after their stretching is a result of increasing spiralization or other processes causing contraction of chromosomes, which in the end overcomes the stretching action of pulling forces. In support of his view he reports observations on autosomal univalents which occur sometimes in crane-fly spermatocytes.

These univalents show no essential changes in length over the whole period of time from the late diakinesis to the late anaphase.

Although DIETZ's hypothesis seems to be rather complicated, it has some unquestionable advantages, viz.: it explains the behaviour of chromosomes in typical mitosis and in monasters as well as the position of multivalents in the equatorial plane in *Ostracoda* (DIETZ 1958). Moreover, it also elucidates cases where considerable elongation of chromosome fibres can be observed, as for example in monopolar mitoses in *Sciara* (METZ 1926, 1933; METZ, MOSES and HOPPE 1926), *Micromalthus* (SCOTT 1936), *Anisolabis* (SCHRADER 1947) or during the metaphase elimination of autosomes in *Brachystethus* and *Mecistorhinus* (SCHRADER 1946a and b, 1947). On the other hand, observations on the behaviour of the bivalents during the prometaphase and metaphase in *Mikiola fagi* seem to disprove some of the tenets of the hypothesis.

The occurrence of the prometaphase stretch of bivalents in the oogenesis of representatives of the subfamily *Cecidomyiinae (Phytophaga celtiphyllia*, WHITE 1950) as well as of *Lestremiinae (Mycophila speyeri*, NICKLAS 1960) seems to indicate that it is no rare phenomenon in *Cecidomyiidae*.

The stretch of the bivalents in *M. fagi* like in other organisms coincides with the appearance of the spindle. The subsequent co-orientation and the movement of the bivalents towards the equatorial plane proceed very rapidly. The bivalents take their definite positions in the equator in the initial period of prometaphase, occupying always the middle part of the spindle. The speed of congression of the bivalents, as well as their central position in the developing plate, might be explained by the fact that they are situated in the middle of the inner part of the prophase nucleus. Observations on the oogenesis in other species of *Cecidomyiidae* (WHITE 1950) confirm the fact that the bivalents assemble in the inner part of the nucleus and take their place in the central part of the spindle at early prometaphase.

The fact that the congression of bivalents during oogenesis in *M. fagi* is not always accompanied by their shortening is, however, the most characteristic feature of the prometaphase behaviour of the bivalents. Simultaneous occurrence in *M. fagi* of both contracted bivalents and more or less stretched ones, also lying in the equator, is characteristic of not only the long prometaphase but of the whole metaphase too. It should be pointed out that the shortening of the bivalents usually does not reach a high degree. Only rarely, as a result of shortening, the bivalents take shapes similar to those they had at late prophase.

An evidence of the fact that the congression of the bivalents towards the equator is not always accompanied by a complete disappearance of the prometaphase stretch is provided by the behaviour of some

bivalents in *Purpura lapillus* (STAIGER 1954), in which they can during oogenesis "auch in später Metaphase noch weit aus der Äquatorialebene herausragen" (p. 423). Furthermore, at the long lasting, stabilized metaphase I during the oogenesis in several species of earthworms (Family *Lumbricidae*, MULDAL 1952), numerous bivalents also show a strong stretch which undoubtedly is a remnant of prometaphase stretch. The behaviour of the chromosomes in these cases, which do not seem to be infrequent exceptions, might be explained by assuming the influence of a modifying factor, the latter being according to DIETZ a deficiency of the chromosomal fibre-forming material. DIETZ assumes that as a result of this deficiency the chromosomal fibre does not attain its maximum length, equal to the half spindle length. Thus, although the elongation of chromosomal fibres in the cases under consideration would lead to the placement of the bivalents in the equator, the above action would not fully compensate for a pulling force arising from the contraction of the fibres. Depending on the deficiency, the action of the force would result in a stronger or weaker stretch of the bivalent which would last until anaphase starts. While accepting the above explanation which seems to be consistent with the DIETZ hypothesis, there is no reason to doubt that both in the above mentioned cases and in the cases where there is no stabilized metaphase plate (e. g. spermatocytes of *Tipula olaracea*, DIETZ 1956), the factor responsible for the action of pulling forces, resulting in the stretch of bivalents at prometaphase and metaphase, is also responsible for the anaphase movement of the chromosomes towards the poles.

Some features of the behaviour of the bivalents at prometaphase and metaphase of the oogenesis in *M. fagi* seem, however, to indicate that there are factors other than the mentioned material deficiency which are responsible for the observed stretch of the bivalents. They are the following ones: (1) a maximum prometaphase stretch of bivalents can frequently be observed after the bivalents have already reached the equatorial plane; (2) the number of the bivalents exhibiting a stretch, both at prometaphase after congression, and at metaphase may vary in different eggs; (3) both at prometaphase and at metaphase the degree of stretching or shortening of bivalents varies greatly, the stretch of the bivalents after congression being greater at prometaphase than at metaphase.

Although identification of individual bivalents in *M. fagi* is impossible at prometaphase, the above observations might indicate that each of the bivalents, after it has occupied its place in the equator, undergoes some alternating stretching and stronger or weaker contraction, which are the result of fluctuations in the strength of pulling forces. The presence of bivalents showing different degree of stretching in the period

of time from early prometaphase through prometaphase until anaphase seems to indicate that there is only one factor responsible for the occurrence of the pulling forces in all the stages. Fixed positions of the bivalents in the equatorial plane might indicate the fluctuations in the strength of pulling forces to be symmetric ones, appearing simultaneously in both chromosome fibres of the given bivalent. On the other hand, it might also be possible that an increase in the strength of pulling force in one of the fibres which involves a slight shift of the bivalent towards one of the poles, brings about a corresponding increase in the pulling force of the other chromosome fibre, thus leading to an increased stretch of the bivalent.

Oscillation of chromosomes between the poles at metaphase (LEWIS 1939; HUGHES and SWANN 1948; HUGHES and PRESTON 1949; BAJER and MOLÉ-BAJER 1956) indicates that the metaphase spindle is not a static system and that the arrangement of chromosomes in the equatorial plate is a result of dynamic equilibrium of forces acting on them rather than of lack of the forces. According to LEWIS such oscillations can be explained by variations in the contractile tension of chromosomal fibres, whereas SWANN (1952) believes them to be a result of "shifts in the precise patterns of orientation in the spindle" (p. 126). When the pulling forces begin to decrease, a corresponding shortening of the bivalent may be a result of the action of elastic forces. It seems, however, that in the period of time from the end of prophase to the first maturation division the bivalents in *M. fagi* continue to contract.

ÖSTERGREN (1951) considers the degree of contraction of bivalents as one of the factors controlling their stretch. According to his hypothesis the disappearance of the prometaphase stretch is a result of an increasing contraction of the chromosomes, the contraction ultimately overcoming the pulling forces acting on the kinetochores and directed towards the poles of the spindle. A confirmation of the first of the above assumptions is provided by STAIGER's (1954) observations on the oogenesis in *Purpura lapillus*. His observations have shown that it is a low degree of condensation of some chromosomes at prometaphase which is responsible for their rather considerable stretch. JOHN and LEWIS (1957) also attribute a strong prometaphase stretch of chromosomes in *Periplaneta americana* to a low degree of their condensation. Thus, a progressive contraction of chromosomes in *M. fagi* might be an explanation for a lesser degree of stretching of bivalents at metaphase and for the occasional occurrence of considerably contracted bivalents. The above phenomena might, however, as well be ascribed to the elongation of chromosomal fibres at metaphase.

Does, however, the contraction of bivalents, following their stretching, really indicate, as DIETZ assumes, a considerable weakening of

pulling forces which at metaphase in the case of considerable contraction of the bivalents may become quite insignificant or even disappear at all? An analysis of the metaphase elimination of the univalents in *M. fagi* oogenesis provides the answer to this question. Before discussing this interesting process a few words should be said about the prometaphase behaviour of the E-chromosomes.

A complete explanation of the more or less random distribution of the E-chromosomes on the spindle surface in *M. fagi* during the initial long period of prometaphase is provided by the results of DIETZ's (1956) observations on movements of the chromosomes at prometaphase of the first maturation division in spermatogenesis of *Tipula lateralis*. As stated by him, at the moment when the spindle is formed, the chromosomes are set in motion between the two poles. „Im Verlauf der Prometaphase ist jedes Chromosom in seiner Bewegung selbständig; der Bewegungsverlauf ist in keiner Weise einheitlich, wie es in der Anaphase der Fall ist. Für das einzelne Chromosom gibt es zu keiner Zeit zwischen dem Beginn des Spindelaufbaues und der Anaphase eine gesetzmäßige Geschwindigkeit oder eine festgelegte Wanderungsrichtung; ebenso willkürlich ist der Zeitpunkt der Richtungswechsel und deren Häufigkeit" (l. c. p. 188). According to DIETZ, only those chromosomes can traverse considerable distances which, while moving at high speed, do not change the direction of their movement over a long period of time. As the probability of such behaviour is very small, only an insignificant number of chromosomes approach the poles. As it follows from statistical analysis, the mean speed of the univalents exceeds that of the bivalents. That is why the sex chromosomes in spermatogenesis of *Tipula lateralis*, despite frequent changes in direction of their movement, approach the poles closer than bivalents.

The fact that univalent E-chromosomes can frequently be observed in close vicinity of the poles, might indicate that they move at a mean speed higher than that of sex chromosomes in *Tipula lateralis*. On the other hand, this might also be a result of relatively rare changes in the direction of their movement. Moreover, the location of univalents at prometaphase at the very spindle poles, which could frequently be observed, seems to contradict DIETZ's assertion that "die prometaphasische Verkürzung der Chromosomenfasern durch einen anderen Mechanismus hervorgerufen wird, als die Verkürzung der Chromosomenfasern in der Anaphase. In der Prometaphase endet die Polbewegung der Chromosomen stets in einem bestimmten Abstand vom Pol, der durch die Länge der kontrahierten Chromosomenfaser gegeben ist. In der Anaphase verkürzen sich die Chromosomenfasern, bis die Chromosomen unmittelbar an den Polen liegen" (DIETZ 1958, p. 426). If it is assumed that the prometaphase movement of chromosomes is a result

of contraction of the chromosome fibres, then polar positions of the univalents at prometaphase would indicate that the fibres are likely to undergo a contraction as strong as at anaphase.

The fact that at late prometaphase the number of chromosomes located near the poles of the spindle decreases, indicates that the E-chromosomes respond to the forces acting in the spindle in a normal way. An increasing concentration of the E-chromosomes in the equatorial area of the spindle seems not to be a result of their slow unidirectional migration towards the equator, but is most likely attributable to the gradual diminution of the area occupied by the moving univalents into a narrow equatorial zone, towards the end of prometaphase, as observed by DIETZ (1956).

Non-random distribution of the univalents belonging to the same chromosome pair was first described by RIBBANDS (1937) in the hybrid *Lilium candidum* × *chalcedonicum*. He found the kinetochores of such univalents to be located on the same arc of the spindle, irrespective of whether they both were on one or on the opposite sides of the equator. A similar regularity was observed by ÖSTERGREN and VIGFUSSON (1953) in *Lilium "testaceum"* and in partially asynaptic plants or plants containing two B-chromosomes of *Secale cereale*. According to the last named authors: "As a rule they are lying on different spindle arcs, but there is statistically significant tendency of these spindle arcs to be more close to one another than expected from random distribution. The univalents thus show an obvious tendency to lie on spindle arcs closely approached to one another" (p. 34).

The occurrence of groupings of the E-chromosomes is a feature characteristic of late prophase in the oogenesis of *M. fagi*. The groups may consist of 2, 3 or 4 chromosomes, which are aligned along the length of the spindle. In connection with RIBBAND's and ÖSTERGREN and VIGFUSSON's findings the question arises whether the formation of the associations can be attributed to a certain homology between these E-chromosomes or whether it can be ascribed to some other factors. The mentioned authors believe the preferential distribution of the univalents on the spindle observed by them to be a relic of a pairing between homologues, which have not formed chiasmata at the preceding prophase. The above explanation cannot be applied to *M. fagi*, since in all prophase stages the E-chromosomes did not show any tendency to pairing. It may be possible that the formation of associations of the E-chromosomes in *M. fagi* at late prometaphase represents a form of delayed somatic pairing between some of these chromosomes. The formation of groups consisting of chains of univalents might also be ascribed to casual meeting of chromosomes moving along the same continuous spindle fibres, or fibres close to one another. Since, however, the frequency of these

associations seems to be too high to be accidental, the above assumption is hardly probable. The question remains unanswered.

Recently, careful consideration has been given to the problem of considerable variations in the behaviour of univalents at meiosis (Östergren 1951; Dietz 1958). One of possible patterns of the behaviour of univalent chromosomes is that initially, while they are showing the monopolar orientation, they lie outside the equator, uniformly distributed on the spindle, and later, with bipolar orientation, they take positions in the equatorial plane. Opinions as to the factor determining the kind of the orientation of a univalent in the spindle are essentially similar. Space arrangement of kinetochores seems most probably to be the factor in question. In the case of strong spiralization, usually shown by chromosomes at meiotic prophase, both kinetochores are located on one side of the chromosome. The monopolar orientation of such univalents depends on the degree of spiralization. If the latter is not high enough to keep the positions of kinetochores towards each other fixed, or if it decreases at prometaphase, the univalent becomes functionally bilateral, gradually undergoes bipolar orientation, and in response to the nature of the forces acting in the spindle, takes its place in the equator. The change from the monopolar to bipolar orientation of univalents has been described in prometaphase, as for example in the case of sex chromosomes in *Tipula maxima* (John 1957a), the X chromosome in *Cyclocypris ovum* (Dietz 1958), or even at anaphase, as in some interspecific *Geum* hybrids (Gajewski 1949, 1957).

Since continuous fibres in *M. fagi* are difficult to distinguish from chromosomal fibres and since spherical univalents are very small, a direct investigation of variations in the orientation of their kinetochores is not possible. Nevertheless, the distribution of the univalents at early prometaphase, their frequent occurrence in the vicinity of the poles, and their more or less equatorial positions at metaphase indicate, undoubtedly, that at late prometaphase the monopolar orientation of the univalents gradually changes to become the bipolar one.

3. Metaphase elimination of the E-chromosomes

Elimination of the univalent E-chromosomes at metaphase is characteristic not only of the oogenesis in *Mikiola fagi*, but it is also met with in other species of the subfamily *Cecidomyiinae* (Kraczkiewicz, unpublished). These are the following characteristic features of the process in *M. fagi:* (1) the elimination is a regular phenomenon occurring in all eggs after the E-chromosomes have occupied positions in the spindle equator; (2) most often all E-chromosomes are eliminated from the spindle; (3) the E-chromosomes present in an egg are eliminated simultaneously; (4) the angle, formed by the direction of movement of the

eliminated univalents with the spindle axis may vary; in extreme cases all univalents move in a direction nearly perpendicular to the spindle axis; (5) the elimination leads to relatively great distances between E-chromosomes and the spindle, which often are considerably greater than the spindle diameter.

Metaphase and prometaphase elimination of chromosomes as a process occurring regularly at definite stages of the chromosome cycle is rare. Elimination of the chromosomes from the metaphase plate, as observed in *Brachystethus* and *Mecistorhinus*, was first described by SCHRADER (1946a and b). In both cases all autosomes forming one aggregate were found to be laterally shifted in the equatorial plane towards the surface of the cell, but during the whole process they were connected with the poles of the spindle with chromosomal fibres. According to SCHRADER (1947, 1953) it is a pushing action exerted on autosomes by the unusually strongly elongating chromosomal fibres that is responsible for the elimination.

The horizontal direction of elimination of the univalents, sometimes observed in *M. fagi*, has only outward resemblances with the autosomal elimination in *Brachystethus* and *Mecistorhinus*. From the lack of any visible fibres connecting the eliminated E-chromosomes with the spindle poles, one may infer that during the elimination the structural bonds between the E-chromosomes and the spindle are severed. In this respect the course of elimination of the E-chromosomes seems rather to be like the ejection of the X chromosome from the spindle, observed in spermatocytes of *Humbertiella indica* (HUGHES-SCHRADER 1948). However, peculiarities of the elimination process in *H. indica* and *M. fagi* should be discussed.

Thus, while the X chromosome in *H. indica* is eliminated into the cytoplasm at early prometaphase during the process of spindle formation without any preceding orientation towards one of the poles, the E-chromosomes in *M. fagi* are eliminated at metaphase, after a long period of normal functioning of their kinetochores. In both cases the chromosome elimination is undoubtedly due to the lack of structural bonds between the kinetochores and the spindle. While, however, the elimination of the X chromosome in *H. indica* is a result of a delayed response of its kinetochore to the developing spindle, the elimination in *M. fagi* is attributable to the simultaneous, temporary subsidence of the kinetochore activity, which activity is responsible for the existence of their bonds with the spindle.

Although in both cases under consideration the lack of the interaction between chromosome and spindle is attributable to different factors, the end effect is the same—the chromosomes are ejected from the spindle. The effect is a striking manifestation of the action of elimination forces

in the spindle (Östergren 1945, 1949, 1951; Östergren, Molé-Bajer and Bajer 1960).

Transverse elimination forces act also in the interzonal part of the spindle at anaphase. This may be inferred from the direction of elimination of the paternal chromosomes during cleavage divisions in the male embryos of *Pseudaulacaspis pentagona* (Brown and Bennett 1957).

At the time immediately after elimination, the E-chromosomes are mostly not in the horizontal plane, but are scattered around the spindle in various planes above and below the equator. If, as Östergren postulates, a chromosome is subjected at metaphase both to the equilibrated pulling forces, acting on their kinetochores in the direction of the poles, and to the elimination force, striving to eject it from the spindle, then the mentioned distribution of the E-chromosomes after their elimination might be explained by assuming that in a chromosome the kinetochores of both chromatids do not lose their activity simultaneously. If such an asynchronous inactivation of kinetochores takes place at the moment when the contact between one of them and the spindle is being broken, the given E-chromosome, subjected to the action of the remaining pulling force, starts moving towards one of the poles. The length of path of the chromosome, hence its place on the spindle surface at the moment of elimination, is a function of a delay in the subsidence in the activity of the other kinetochore; that is why the E-chromosomes immediately after their elimination from the spindle into the cytoplasm occupy different positions in relation to the equatorial plane.

The fact is worth noting that a few E-chromosomes which might not have been eliminated and remain in the spindle, change their positions. They are still located on the spindle surface, but shifted towards one of the poles. This fact might be ascribed either to weakening of the bond or to an interaction between the spindle and one of the kinetochores, caused by a partial inactivation of the latter.

Hughes-Schrader considers a cytoplasmic current in the equatorial zone of the cell to be the factor responsible for the considerable distance between the eliminated X chromosome and the spindle in *H. indica*. Since the E-chromosomes in eggs of *M. fagi* are frequently ejected over a greater distance from the spindle than the X chromosome in *H. indica*, one would infer that either the eliminating force is relatively large in this case or a more intense cytoplasmic current occurs around the equatorial part of the spindle.

The cytoplasmic currents, according to Hughes-Schrader occurring at mitosis, arise most probably as a results of surface tension differences, and, although they appear prior to the division itself, they undoubtedly are related to the mode of cytokinesis of animal cells. They are always

observed in the cases when the division of the cell is the result of furrow formation, usually in the equatorial plane. However, to assume such currents in eggs of *M. fagi* during the elimination of the E-chromosomes seems hardly probable for the following reasons: (1) maturation of *M. fagi* eggs, like those of other dipteran flies, is not accompanied by cytokinesis; the polar body (a nucleus) remains within the egg cytoplasm; (2) elimination takes place long before the maturation divisions; (3) elimination is not affected by the orientation of the spindle with respect to the egg surface, and thus, the current direction would depend solely on the position of the spindle axis. This seems improbable. It is possible that in the cases when mitosis in an animal cell is not accompanied by cytokinesis, regular cytoplasmic currents at the spindle appear only at the beginning of anaphase, similar as in plant cells (BAJER and MOLÉ-BAJER 1956).

Taking the above facts into account, the conclusion seems justified that cytoplasmic currents cannot be the principal factor responsible for the considerable distances between the eliminated E-chromosomes and the spindle. It must be the motion of the chromosomes under the influence of a centrifugal force manifesting itself after the bonds between the kinetochores and the spindle have broken. This causes the displacement. The problem of the influence of these two factors on the movement of E-chromosomes in the cytoplasm immediately after their ejection from the spindle is, however, of minor importance. The existence of transverse elimination forces in the spindle as postulated by ÖSTERGREN is beyond doubt.

As it follows from HUGHES-SCHRADER's observations, the elimination forces in the spindle start acting already at early prometaphase. The elimination of E-chromosomes at full metaphase clearly indicates that the forces do not disappear during prometaphase. How then should the fact be explained that the bivalents which at early prometaphase take positions in the middle of the equatorial plane of the spindle do not change them later? At prometaphase and at metaphase stretched bivalents as well as contracted, and sometimes considerably contracted, bivalents can be observed. If the contraction of the bivalents were brought about by the growth of chromosomal fibres, which is accompanied by a relaxation of pulling forces, a contracting bivalent in the plate under the action of elimination forces would be gradually displaced and would move towards outer parts of the spindle. Since, however, the contraction of the bivalent is not accompanied by its displacement the existence of a force compensating for the centrifugal elimination forces should be assumed. Only forces pulling the kinetochores towards the poles seem to be the forces in question. Thus, the contraction of bivalents is not an absolute indication of the disappearance of pulling forces and the hypothesis assuming chromosomal fibres

not, or nearly not, to exert an action on the kinetochores at metaphase is not of such a general significance as DIETZ believes.

Any more serious attempts to interpret the E-chromosome behaviour in the period of time after the elimination would be premature because of its abnormal character and since for the time being any material for comparison is lacking. What is the force that sets the E-chromosomes scattered in the cytoplasm in motion, and then brings them together to form one or two groups under the egg surface, close to the spindle containing the bivalents? Since distances between single E-chromosomes are great, the possibility that their interaction during initial stages of their migration towards the egg surface may play some role seems hardly probable. Although, as mentioned before, any intense cytoplasmic current in the equatorial plane of the spindle seems unlikely, it may be possible that there are some currents at the boundary between the outer and inner cytoplasm layers which are directed towards the egg surface. If so, a very slow migration of the E-chromosomes towards the egg surface, unlike their fast movement during their elimination, might suggest that the currents are weak ones. While the migration of the E-chromosomes in some general direction may be ascribed to the above currents, it seems absolutely improbable that it is the currents only that are responsible for the grouping of the widely scattered chromosomes to form one or two compact groups.

The fibrillar structures which are formed at each group of E-chromosomes do not differ from a normal spindle. During the division of E-chromosomes in other species of *Cecidomyiidae* (KRACZKIEWICZ, unpublished) their functioning clearly indicates that they are spindles. Of all cecidomyiid species investigated so far only in *M. fagi* these spindles are transitory structures not serving in the destribution of E-chromosomes. Shortly before oviposition the E-chromosomes again join the spindle which contains the bivalents. The author, however, did not succeed in tracing this process in detail.

In the stage preceding maturation divisions, the univalents which are now once again in the spindle together with the bivalents are only rarely located in the equatorial plane. Most often groups of E-chromosomes are seen to adhere to the spindle surface at different distances from the equator. The shifting of the univalents towards the poles indicates their orientation to be monopolar. The change in the orientation, as compared with the one shown before the elimination, should most probably be ascribed to the increasing spiralization of the E-chromosomes. In each of the groups, the E-chromosomes are arranged in chains which are located along the spindle. However, similarly to conditions in prometaphase, the factor responsible for such arrangement has been difficult to determine.

4. Maturation divisions

The anaphase separation of chromosomes is usually a result of two factors: the migration of kinetochores towards the poles and the increase in the distance between the poles which are linked to the kinetochores with chromosomal fibres. In some cases the anaphase movement of chromosomes is due to the second of the two factors solely (RIS 1943; DIETZ 1954, 1958). Opinions agree on the whole that the movement of chromosomes towards the poles is a result of the action of a force, exerted at the kinetochores, which is most probably conditioned by some properties of the chromosomal fibres. The problem, however, of the source of the pushing forces, which set the poles in motion, as well as the mode of their action, are still subjects of controversy. Thus, the movement of the poles is ascribed either to the elongation of the whole spindle (RIS 1943, 1949; DIETZ 1954, 1958) or to the elongation of its interzonal part only (e. g. BĚLAŘ 1929; CARLSON 1952, 1956; BOSS 1954).

The interzonal part of the spindle undergoes a considerable liquefaction at anaphase (CARLSON 1952). There are indications, however, that its pushing action increases with the liquefaction (BAJER 1953). Another characteristic phenomenon observed at anaphase is the fall in birefringence of the interzonal part of the spindle (SCHMIDT 1939; HUGHES and SWANN 1948; INOUÉ and DAN 1951; INOUÉ 1953; SWANN 1951; URETZ after ZIRKLE 1959). On the basis of the above findings CARLSON (1952, 1956) infers that the lengthening of interzonal connections is responsible for the anaphase elongation of the spindle. The transformations of the spindle during the first maturation division in *Mikiola fagi* cannot, however, be explained by the action of interzonal connections.

At anaphase I, which is characterized by a strong elongation of the spindle, the interzonal connections would possibly arise only between separating S-chromosomes. Even assuming the existence of the interzonal connections in *M. fagi* and their role in the elongation of the spindle as a pushing body (Stemmkörper), they would be expected to cause elongation of the middle part of the spindle only in conformity with the position of the bivalents. But, in fact, it is the whole spindle that is elongated, both its inner and its outer part, the latter having the E-chromosomes on its surface which most frequently do not divide at that time. Thus, at least as far as the elongation of the outer part of the spindle is concerned, it is not due to the elongation of interzonal connections. The interzonal connections in *M. fagi* seem not to play the role assumed by CARLSON for divisions of neuroblasts in *Chortophaga viridifasciata*. The present author believes it to be most probable that the increase in the distance between the poles during the first maturation

division in eggs of *M. fagi* is brought about by the elongation of continuous fibres.

It is noteworthy that at anaphase the birefringence of the interzonal part of the spindle both in animal and plant cells does not disappear completely. This might indicate that the original, fibrillar structure of the spindle has partially been retained in it. According to HUGHES and SWANN (1948) "This residual birefringence in the spindle presumably corresponds to the continuous spindle fibres of various authors" (p. 67). The assumption has been confirmed by observations on the oocytes of *Chaetopterus pergamentaceus* and on PMC of *Lilium longiflorum*, where continuous, birefringent fibres could be seen both at metaphase and anaphase (INOUÉ 1953).

Towards the end of anaphase I, the S-chromosomes dyads are no longer in contact with the elongated spindle which from this moment on will serve in the regular transport of the daughter E-chromosomes.

The movement of the E-chromosomes at early anaphase I is mostly due to the elongation of the spindle. Since the distribution of E-chromosomes on the spindle before the anaphase is irregular, it is difficult to determine if simultaneously with the spindle elongation their positions with respect to the poles change. If the chromosomes do move towards the poles at this stage, their movement is probably very slow. This movement seems to be accelerated only at the time when the contact between the S-chromosomes and the spindle is being lost, since at metaphase II some of the then dividing E-chromosomes lie near the poles of the first meiotic spindle. At the end of a considerably delayed anaphase movement, the E-chromosomes which have behaved as univalents being oriented towards one of the poles, undergo the equational splitting.

Equational splitting of the E-chromosomes is accompanied by a striking change in their orientation in the spindle, namely, the long axes of the daughter E-chromosomes shift parallel to the spindle axis and retain this orientation for at least the initial period of anaphase movement. From the fact that during this movement the daughter E-chromosomes are directed towards the poles with one of their ends instead of with the kinetochore, one might infer that a neo-centric activity of the chromosome ends appears. In dipteran flies such an activity of chromosome ends has recently been postulated at meiosis in *Tipula maxima* (JOHN 1957a).

The equal number of chromosomes in both groups of the separating E-chromosomes indicates that the two halves formed by the equational splitting of each E-chromosome move towards the opposite poles of the spindle. The most characteristic feature of the movement, besides the positions of the chromosomes, is that the location of an E-chromosome on the spindle before its division does not affect the regular distri-

bution of its daughter chromosomes between the two poles. In *M. fagi* the division of the E-chromosomes scattered over the spindle surface might be compared to the case when the anaphase movement of daughter chromosomes starts at prometaphase hence is not preceded by the formation of metaphase plate. During the anaphase, some daughter E chromosomes are observed to migrate towards the pole opposite to the one they have been situated at just before initiation of their anaphase movement. It is possible that it is the continuous fibres that facilitate the contact of the chromosome with the distant pole. Although the assembly of daughter E-chromosomes in the later stages of their anaphase movement to form two relatively compact groups is due to some other factor which is difficult to determine, it seems that frequently observed irregular or strongly elongated shapes of the groups might be ascribed to irregular distribution of the E-chromosomes on the spindle surface during their equational division.

II. Oogenesis evolution in the subfamily *Cecidomyiinae*

Recently, NICKLAS (1960) described a substantial part of the chromosome cycle in the cecidomyiid *Mycophila speyeri* belonging to the primitive subfamily *Lestremiinae*. The number of chromosomes in germ-line cells of *M. speyeri* is 29. In somatic cells of ♀ and ♂ there are 6 and 3 respectively. The difference is due, as in other *Cecidomyiidae* species, to the sex specific elimination from future somatic nuclei of a number of chromosomes. NICKLA's observations on the oogenesis in sexual females have brought striking and remarkable results. Thus, both at diakinesis and metaphase I cells commonly contain 14 bivalents and one univalent. Both maturation divisions proceed in a typical manner. At anaphase I the univalent undergoes random segregation, whereas it divides equationally at anaphase II.

The insight into the course of oogenesis in the representative of the primitive subfamily *Lestremiinae* and in more specialized cecidomyiids as well is an incentive for expressing views on evolutionary changes in oogenesis in bisexual generations of species belonging to the subfamily *Cecidomyiinae*.

Assuming the oogenesis in the subfamily *Cecidomyiinae* to proceed in its initial form similarly as in *Mycophila*, the first modification of the oogenesis in this subfamily seems to be the loss of the ability of E-chromosomes to pair at meiosis, i. e. to form bivalents. This process most probably has proceeded by steps, extending gradually to all E-chromosomes. The form of oogenesis where all E-chromosomes occur as univalents seems to be the last stage of the process. This form, first described by WHITE (1950), is characteristic for all species of the subfamily *Cecidomyiinae* that have been investigated so far. In *Ceci-*

domyiidae the first signs of an evolutionary tendency to increase the number of univalents appear in *M. speyeri*. In the scanty material which NICKLAS had at his disposal he was also able to observe oocytes containing 3 univalents and 13 bivalents.

The course of prophase in the oogenesis of *Mikiola fagi* indicates that in a certain evolutionary stage of the chromosome cycle in *Cecidomyiidae* a new feature of E-chromosomes appears in form of a delay in the mitotic cycle of E-chromosomes as compared with the mitotic cycle of S-chromosomes. Such a delay has also been observed in *Phytophaga celtiphyllia* and *Oligotrophus pattersoni* (WHITE 1950). Since information on early prophase of the oogenesis in *M. speyeri* is lacking, there is no possibility to prove that an analogous delay occurs in the *Lestremiinae* also. Even if it is so, it would remain uncertain whether its first signs appeared prior to the loss in the pairing ability of E-chromosomes or whether they appeared only after this process had started. However, an indirect indication is provided by the behaviour of the univalent chromosome in *M. speyeri* during the first maturation division. Its shift towards one of the poles at metaphase and its random segregation at anaphase indicate its monopolar orientation resulting from the univalent nature of the chromosome. Thus, if a delay in the univalent mitotic cycle at prophase does take place it seems not to be so severe as in other cecidomyiids and it seems to be overcome towards the end of prophase.

In species with an insignificant delay in the mitotic cycle of univalent E-chromosomes, the latter are assumed to have divided after the pattern discovered in *M. speyeri*, while in other species as a result of a considerable delay in their mitotic cycle the sequence of the events might be reverse[1]. Regardless of the pattern of univalent behaviour it was the increase in the number of univalent E-chromosomes (with a simultaneous decrease in number of bivalents) which has probably led to their gradual loss during oogenesis. Since retention of E-chromosomes in the germ-line nuclei seems to be an indispensable condition of a normal course of oogenesis (GEYER-DUSZYŃSKA 1959; BANTOCK 1961), the process of elimination of the univalent E-chromosomes from the chromosome cycle or, to be more exact, the decrease in their number in a population must have been counteracted by a selection as in the case of supernumerary X chromosomes in *Cimex lectularius* (DARLINGTON 1939). Under these circumstances the selection is expected to fix any oogenetic modification which would lead to the stabilization of the highest possible number of E-chromosomes.

[1] Since the univalents prior to their elimination are located together with the bivalents in the equatorial plane one may infer that *M. fagi* probably descended from a form in which the majority of univalent E-chromosomes had undergone equational division at anaphase I.

The evolution of oogenesis in *Cecidomyiinae* involves the appearance of a new, hitherto not observed mechanism which prevents a decrease of the number of univalents. This mechanism consists at first in the elimination of the univalent E-chromosomes from the spindle, and then in the formation of a new spindle around the eliminated chromosomes. The changes which have led to this mechanism must have been progressive ones, involving increasingly greater numbers of E-chromosomes. This may be inferred from the fact that besides *M. fagi*, where most frequently all of the E-chromosomes are eliminated, other species of the subfamily *Cecidomyiinae* have been found in which the number of eliminated E-chromosomes may vary within wide limits (KRACZKIEWICZ, unpublished).

What, however, is the cause that the above mechanism is not always utilized? In *M. fagi*, and probably in other related species too, a new interesting modification of oogenesis appears. The modification consists in the return of the E-chromosomes on the spindle from which they were previously eliminated. This process of undoubtedly secondary character is caused by various changes in the properties of the E-chromosomes. Thus, the union of the E-chromosomes with the spindle containing the bivalents does not restore the state before the elimination. This can be inferred from the positions of E-chromosomes with respect to the spindle equator which mostly differ from those before the elimination. The behaviour of E-chromosomes in *M. fagi* during the first maturation division, in comparison with that of E-chromosomes in other species of *Cecidomyiinae*, is another indication of changes in their properties. Thus, while the anaphase movement of a number of the E-chromosomes in *Rhabdophaga saliciperda* (KRACZKIEWICZ, unpublished), both in the spindle containing the bivalents and in the one formed by the eliminated chromosomes usually takes place simultaneously with the anaphase movement of the S-chromosomes, the movement of E-chromosomes in *M. fagi* starts much later. Some correlation, however, exists not only between the return of the E-chromosomes on the spindle and the delay in their anaphase movement but also between the former and the equational division of the E-chromosomes at the end of anaphase I and the specific mode of functioning of the first meiotic spindle. That is why despite the return of the E-chromosomes on the spindle containing the bivalents, the mode of clonal transmission of the E-chromosomes from generation to generation by females, guarantees their continuous presence in germ-line nuclei in considerable numbers.

NICKLAS's (1960) studies on *M. speyeri* have shown that the modification of spermatogenesis, typical of all cecidomyiids, is more primitive

than any modification of oogenesis. The sperm of *M. speyeri* contains 3 chromosomes. On the basis of his observations on oogenesis in *M. speyeri*, cited above, NICKLAS has come to the conclusion that in addition to fertilization some compensatory mechanism seems to function in this species which controls the number of germ-line chromosomes in embryos. It may, however, also be possible that fertilization in *M. speyeri*, if it takes place at all, is of no genetic significance and that the number of chromosomes in eggs is regulated as in a case of meiotic parthenogenesis. HAUSCHTECK's studies (1959) on the paedogenetic origin of males in *Oligarces paradoxus* have proved the existence of such a form of parthenogenesis in *Cecidomyiidae*. The reconstruction of the chromosome cycle in *M. fagi* from oogenesis is less difficult than in the case of *M. speyeri*. Should, however, the fertilization in *Cecidomyiidae* be considered as a prerequisite for normal development in bisexual generations ? In this respect facultative parthenogenesis occuring in *Wachtliella persicariae* (NIJVELDT 1951), a typical bisexual species, is worth attention. Observations on the sex ratio in *M. fagi* (MATUSZEWSKI, unpublished) also shed some light on the occurrence of facultative parthenogenesis in *Cecidomyiidae*. The percentage of males in populations occurring in the Polish Tatra is about 0.1% as compared with 20% in a population in the vicinity of Copenhagen (BOYSEN HENSEN 1948) and with about 10% in a population in the environs of Brussels. The conclusion seems sound that in the Tatra populations of *M. fagi* the biological role of males in reproduction is quite insignificant and that most of the females lay unfertilized eggs which then develop parthenogenetically.

The mentioned occurrence in *M. fagi* of unfertilized eggs containing two prophase or prometaphase nuclei each with the same number of chromosomes might indicate that in the case of parthenogenetic development, the first cleavage division nucleus is formed by fusion of the egg nucleus containing 4S- and 16 E-chromosomes, with one group of 4 S-chromosomes which has arisen as a result of the second maturation division.

III. Origin of the mitotic spindle

It seems reasonable to agree with the view expressed by MAZIA (1960) that the speculations on the problem of "nuclear versus cytoplasmic origin of the mitotic spindle" are of value for each given case only. Thus, in addition to a category of spindles originating from cytoplasmic proteins, as in sea urchins (MAZIA and ROSLANSKY 1956; KAWAMURA and DAN 1958; WENT 1959), there is undoubtedly a category of spindles which are formed in nuclei before disappearance of the nuclear membrane, or just during this process, from material accumulated in the

nuclei before the division (e. g. the first meiotic spindle in eggs of *Cyclops strenuus:* STICH 1952; STICH and MCINTYRE 1958).

On the basis of morphological criteria, the spindle formed in the oogenesis of *Mikiola fagi* at prometaphase of the first maturation division should be regarded as of nuclear origin. In this case, however, it is not the whole nucleus that is transformed into the spindle. Only a part of the nuclear sap, forming the inner zone of the nucleus and containing at the end of prophase all the chromosomes, is used for the construction of the spindle. Optical properties (MATUSZEWSKI, unpubl. observations on living material), the mode of staining as well as mechanical properties after fixation indicate that the substance which occupies the middle part of the nucleus is less hydrated than the rest of the nuclear sap and is, most probably, of a gel nature. The substance appears already at early prophase. Later it occupies, together with the chromosomes the middle part of the nucleus. An analogous differentiation in the nucleus has also been observed for example at the end of the oocyte growth in various amphibians (WAGNER 1923; DURYEE 1950; CALLAN 1952). Also here, as in the oocytes of *M. fagi,* all chromosomes collected in the middle of the nucleus are immersed in a part of karyolymph differing in its properties from the rest of the nuclear sap. The above analogy is still more remarkable, as according to WAGNER and DURYEE the first meiotic spindle is made of the gel contained in the inner part of the nucleus. In connection with this, the fact is noteworthy that the nuclear sap of amphibian oocytes contains substantial quantities of proteins rich in —SH groups (BRACHET 1960).

In a number of cases when spindles are of nuclear origin, spindle-forming substance can be observed on the surface of chromosomes (e. g. *Saccocirrus:* BAEHR 1920; *Acroschismus:* HUGHES-SCHRADER 1924; *Moina:* DEHN 1948) similarly as in the oocytes of *M. fagi.* Since this is a regular phenomenon, the question arises whether the substance is produced by the chromosomes or whether it is formed in the nucleus without their direct participation in the process. Since, in the oogenesis of *Cyclops,* RNA appears and accumulates in the nucleus prior to the increase in the content of nuclear proteins, which are the material for the spindle formation, STICH and MCINTYRE (1958) suggest that besides the nucleolus and the chromosomes there is a third protein synthesizing system in the nuclear sap. If it were so, and if its presence were characteristic of other cases of the nuclear origin of the spindle, then the accumulation of the spindle-forming substance around the chromosomes might be explained as a result of its gradual adsorption on the chromosome surface. The results of SATO's (1960) electron microscopic observations on the spindle formation in PMCs of several species of *Lilium* speak in favour of STICH's hypothesis. When studying the course

of the prophase the author observed a granular substance to appear on the inside of the nuclear membrane which later, as its quantity increased, becomes dispersed in the nuclear sap and deposited on the surface of the chromosomes. Towards the end of prophase in place of this material there arise short fibrils, from which, as SATO believes, spindle fibres are formed. The process of the formation of chromosomal fibres starts at the kinetochores and then gradually spreads towards the poles.

In the case of *M. fagi* the adsorption of the spindle-forming material at the chromosome surfaces should be considered as highly specific, since in the initial period of the formation of the inner part of the nucleus, the material (perichromosomal substance) accumulates around the S-chromosomes solely, and not on the surface of the E-chromosomes.

The other alternative seems, however, not to be impossible. Recent investigations on plant material with the use of the interference microscope have shown that chromosomes may more directly participate in the production of spindle-forming material. Thus, RICHARDS and BAJER (1961) observed a decrease in the total dry mass of the nucleus at prophase, which means that in this period some nuclear material penetrates the cytoplasm. The authors assume this to take place simultaneously with the formation of the so called clear zone around the nucleus, which is the first sign of spindle formation (BAJER and MOLÉ-BAJER 1956; BAJER 1957). The dry mass of chromosomes per unit area which gradually increases at prophase, decreases considerably during the formation of the clear zone (AMBROSE and BAJER 1961). Since this is not accompanied by a strong contraction of the chromosomes, the authors infer that some material is given off by the chromosomes which participates in the formation of the clear zone and presumably of the spindle too.

The formation of additional spindles, containing chromosomes which, for some reason or other, have lost the initial contact with the original spindle, is a phenomenon frequently observed during microsporogenesis in interspecific plant hybrids. Recently, KOOPMANS (1958) has advanced a view that the formation of such spindles might be explained by assuming the existence of a stable structural bond between the kinetochore of a chromosome and a certain part of the mitotic centre (RESENDE 1947; LETTRÉ and LETTRÉ 1957; RESENDE, LETTRÉ and LETTRÉ 1959). Observations on elimination of the E-chromosomes in *M. fagi* as well as on their behaviour in the cytoplasm do not confirm this rather improbable hypothesis.

In the oogenesis of *M. fagi* the number of groups formed by the eliminated E-chromosomes, around which additional spindles can be observed, seems to be accidental. Thus, it seems very likely that the spindle

are formed without the participation of any mitotic centres. As the spindles appear only after a certain lapse of time, after the eliminated E-chromosomes have formed groups, it is possible that the spindles are organized by the E-kinetochores out of some cytoplasmic material. Since in other species of *Cecidomyiidae*, in such additional spindles which are formed in an analogous way, chromosome movement proceeds, as it has been mentioned above, in quite a normal way, it may be assumed that the structural and functional bipolarity of the spindles is a result of physico-chemical properties of the material they are made of.

The conclusions fully agree with the results of observations on the formation and functioning of the spindles in spermatocytes of *Pales crocata*, which have been either flattend out, or subjected to thermal shock or treated with a hypotonic sugar solution (DIETZ 1959). In such spermatocytes bipolar spindles can be formed without participation of one or even both centrosomes. This has, however, no effect on the formation of a metaphase plate and on the anaphase movement of the chromosomes. Thus, chromosomes move towards both centric and acentric poles in a normal way, and at nearly equal speeds. DIETZ infers that in tipuline spermatocytes spindles are organized mostly by the chromosomes and the activity of centrosomes is of only minor importance and is limited to focussing the spindle poles.

Summary

1. Female somatic nuclei of *Mikiola fagi* contain only diploid sets of S-chromosomes (8 S), while germ-line nuclei have 24 chromosomes (8 S-chromosomes + 16 E-chromosomes).

2. In oogenesis only S-chromosomes form chiasmatic bivalents, while E-chromosomes occur as univalents. At prometaphase, and immediately before the first maturation division, the E-chromosomes may temporarily form associations of two, three or four, a contact which is of the somatic pairing type.

3. At metaphase, E-chromosomes are simultaneously eliminated from the spindle. This phenomenon is probably the result of a temporary inactivation of the kinetochores of E-chromosomes and may be interpreted as proof of the existence of transverse elimination forces in the spindle.

4. The E-chromosomes eliminated from the spindle aggregate as a rule in one or, less frequently, two or three groups, each of which forms then its own spindle.

5. Shortly before the first maturation division E-chromosomes rejoin the spindle with the bivalents.

6. During the first maturation division, which is a reduction division for S-chromosomes, the anaphase movement of E-chromosomes is

greatly delayed. E-chromosomes, owing to the strong anaphase elongation of the spindle, are passively distributed on its surface.

7. There is no interphase between the first and second maturation division. Towards the end of anaphase I two small spindles of the second meiotic division are formed at the spindle poles, each of which contains only one haploid set of S-chromosomes.

8. Equational splitting of E-chromosomes occurs mostly when the S-chromosomes are at metaphase of the second maturation division. The anaphase movement of daughter E-chromosomes begins without prior formation of a metaphase plate. The position of E-chromosomes on the spindle surface seems to have no influence on the separation of the daughter chromosomes and their migration to the opposite poles of the first meiotic spindle. The anaphase movement of the daughter E-chromosomes, which occurs at the time when S-chromosomes undergo the second maturation division, leads to the formation of two groups each containing 16 chromosomes. In the early stages of this movement, each of the daughter E-chromosomes is oriented towards the pole not with its kinetochore but with one of its ends.

Thus, the bivalent S-chromosomes undergo two maturation divisions whereby their number is reduced, while the univalent E-chromosomes undergo only one equational division.

9. It is postulated that the egg nucleus is formed as the result of a fusion between one haploid set of S-chromosomes and one set of E-chromosomes. As it is not known whether the eggs develop without fertilization, the establishment of the diploid number is open to discussion. Probably, in the Polish populations of the species with a very low percentage of males, the development starts with the fusion of the egg-nucleus with a haploid set of S-chromosomes whereby the number is raised from 20 to 24.

10. The evolution of cecidomyiid oogenesis is discussed. The modifications of oogenesis observed in the subfamily *Cecidomyiinae* are interpreted as mechanisms preventing the decrease of the number of univalent E-chromosomes during maturation divisions.

11. During prophase a special nuclear material accumulates around the chromosomes grouped inside the nucleus and leads to its characteristic differentiation into an interior part containing both S- and E-chromosomes and an exterior zone of nuclear sap. In prometaphase the first meiotic spindle arises from material localized in the interior part of the nucleus. The problems of the origin of the spindle-forming material as well as the formation of spindles by the E-chromosomes eliminated into the cytoplasm are discussed.

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Dr. BOHDAN MATUSZEWSKI,
Zakład Cytologii, Uniwersytet Warszawski,
Warszawa 64, Krakowskie Przedmieście 26/28, Polska

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BREAKDOWN OF DIVISION CYCLE AND ORGANISATION OF ATYPICAL SPINDLES IN FUSED POLLEN MOTHER CELLS OF LOLIUM*

By

H. K. JAIN

With 6 Figures in the Text

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A. Introduction

Perennial rye grass *(Lolium perenne)* is adapted to grow in temperate regions. The extreme sensitivity of pollen mother cells in several of its clones to high temperature conditions has proved very useful in analysing a number of chromosomal changes and functions during meiotic division. The temperature induced conditions which have made this analysis possible include correlated failure of nucleolar and DNA synthesis in the chromosomes, adventitious nucleolar formation by the different chromosomes at the expense of their normal centromeric activity, and neo-centric reaction in the terminal parts of bivalents (JAIN 1957, 1958, 1960a, 1960b). The present paper relates to breakdown of division cycle and organisation of abnormal spindles in the plasmodial pollen mother cells of a different clone subjected to similar high temperatures.

B. Method

Plants of a clone which formed part of material for genetical studies were exposed to high temperature of 34 ± 3^0 C during their flowering period. The treatment given for 72 hours was so timed that at the end of it, a number of spikes showing pollen mother cells at different stages of meiotic division were available for fixation. The method of temperature treatment and cytological analysis has been described earlier (JAIN 1957).

C. Observations

1. Formation of plasmodial masses

The different pollen mother cells in the control material appear quite distinct and show normal meiotic behavior (Fig. 1). Following the organisation of typical bipolar spindles, the 7 bivalents have the chromosomes separating into two convergent groups and with the completion of the succeeding mitotic division, viable pollen grains are obtained. The disturbed course of meiosis in the treated plants could be traced to the failure of wall formation in the pollen mother cells as a result of which

* These studies were undertaken at the Department of Agricultural Botany, University College of Wales, Aberystwyth, and I am grateful to Prof. P. T. THOMAS for helpful advice.

they fuse into plasmodial masses of varying size (Figs. 2, 4). A number of such masses occur in the anthers; in some of them the faint outlines of individual cells could be still recognised. Except for the failure of wall formation in the resulting pollen mother cells, the premeiotic mitotic

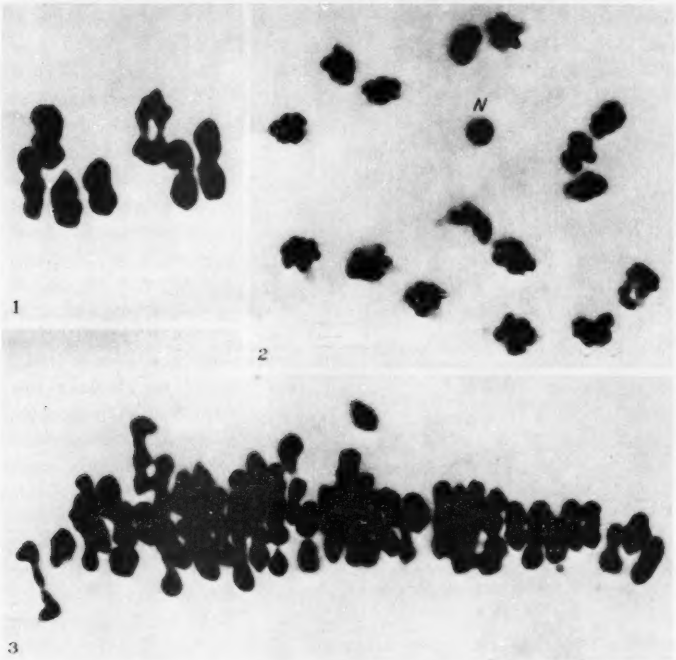

Fig. 1. Pollen mother cell showing 7 bivalents on a metaphase plate (control material). × 1680. — Fig. 2. A plasmodium showing 16 out of a total of 17 distinct nuclei, each with 7 clumped bivalents in the diakinesis stage. A single nucleolus (*N*) can also be observed in many of them. × 225. — Fig. 3. Oversize methaphase plate with the bivalents showing normal orientation × 970

divisions give every indication of having been normal, for the nuclei in the cytoplasmic masses with their intact membranes appear similar to those in the cells of the control material and enter the meiotic prophase with characteristic regularity.

2. Oversize metaphase plates and divergent anaphase separation

The prophase development of chromosomes in different nuclei in a plasmodium is found to be perfectly synchronised. This is as much evident at early prophase as at diakinesis, with all the bivalents showing

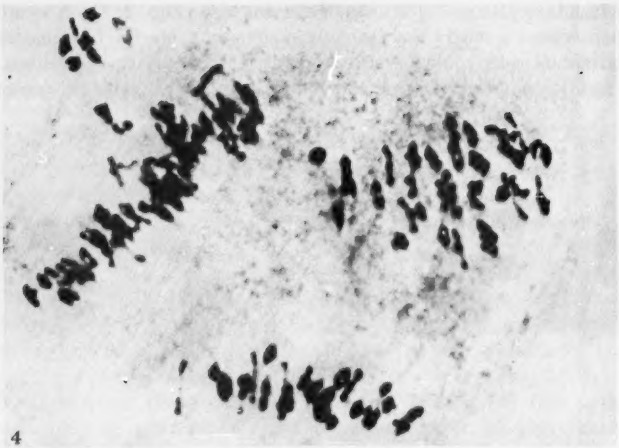

Fig. 4. Four metaphase plates of varying size in a plasmodium. The one at the upper left corner is of normal size with only 7 bivalents. × 600

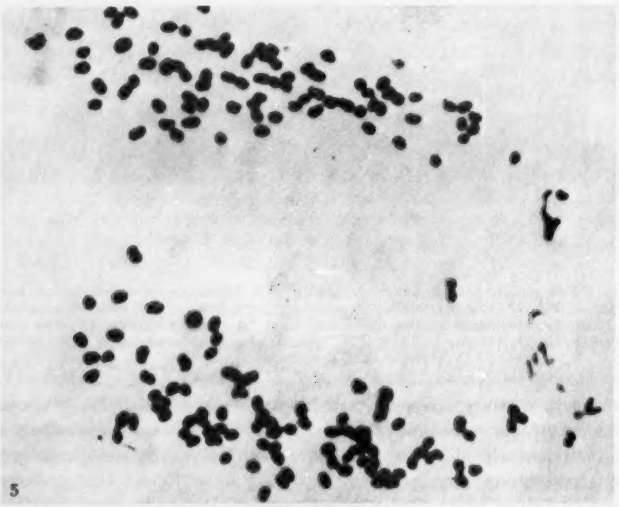

Fig. 5. Anaphase-I separation of divergent type from an atypical spindle. × 530

uniform condensation (Fig. 2). A single nucleolus can also be seen in each of the nuclei.

The barrier which keeps the different nuclei separate from one another in the common cytoplasm disappears with the beginning of metaphase. Following the dissolution of the nuclear membrances, the bivalents from different nuclei arrange themselves on a common metaphase plate in many of the plasmodia. In other words, a single giant spindle is organised in these (Fig. 3). In others, several spindles may be organised which vary in size, some having as many as 56 or more bivalents on their equatorial region, others as few as 7, the normal number (Fig. 4). The co-orientation of bivalents on all the spindles including the very large ones, is found, as a rule, to be very regular. Apart from the increase in the size of the equatorial region, an error in the organisation of the achromatic figures manifests it self at anaphase when the bivalents are found to show divergent type of separation. The separated chromosomes, instead of converging to two poles as in the control plants, are distributed in lines running parallel to the metaphase plate (Fig. 5). This lengthwise distribution of the chromosomes clearly indicates that the "spindles" are without well defined poles. They are probably as wide in their terminal parts as in the middle. The widely scattered anaphase chromosomes fail to be included into a single telophase nucleus. Instead, a large and several smaller nuclei of differing size are constituted. The cytoplasm between the two groups of chromosomes divides regularly but this is not followed by the formation of cell walls.

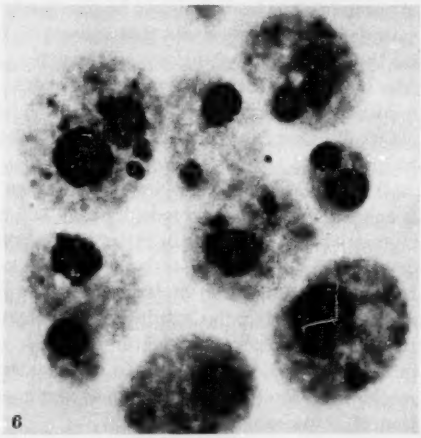

Fig. 6. "Pollen grains" varying in size, each with two or more degenerated nuclei. × 365

3. Breakdown of nuclear cycle

The breakdown of meiotic division in most plasmodia occurs at this stage. The telophase nuclei appear highly pycnotic showing obvious signs of degeneration. They do not proceed further with the nuclear cycle. The very few nuclei which enter second division show a regular mitotic metaphase but the chromosomes undergo a similar disorganisa-

tion of the chromatin material soon after their anaphase movement. Thus, no viable pollen grains are formed; they are replaced by cells which are extremely variable in size, having two or more pycnotic nuclei (Fig. 6).

D. Discussion

The occurrence of plasmodial pollen mother cells in a number of plants has been described previously by several authors including LEBDEFF (1940), SMITH (1942) and SNOAD (1954). In all these cases, the abnormality has been shown to be genetically determined. In the present material there is little doubt that a direct temperature effect is responsible for the plasmodial condition and the genotypic factors are of importance only in so far as they determine the response of the clone to temperature treatment. This genotypic importance is indicated by the fact that plants of other clones similarly treated failed to respond in an identical manner.

The course which meitoic division takes in a plasmodium may vary depending upon its size, mode of origin and other factors. In the present material it corresponds, in its pre-telophase stages with the sequence described by SMITH (1942) in the so-called multiploid sporocytes of barley. The formation of large size metaphase plates and the diffuse nature of spindle poles resulting in divergent type of anaphase separation, are the most striking features of this sequence. They are of obvious interest in relation to the mode of organisation of the spindle and the mechanism determining metaphase arrangement and anaphase separation of chromosomes.

The organisation of spindles is generally believed to be a result of orientations produced by the centrosomes, whose role is obvious and the centromeres whose contribution is not so obvious (SWANN 1952). SWANN has also offered an explanation for the typical form of the achromatic figure. He has suggested that when the two regions of orientation, initiated by the two centrioles, meet, the two lines of orientation would bend round and arrange themselves parallel which would necessarily produce a spindle shape. The formation of atypical spindles in the plasmodial masses provides support both for the important role of centromeres in organising these bodies and of the centrosomes in determining their form. It is clear that the organisation of giant spindles in the present material and, by analogy, in the multiploid sporocytes of SMITH, is brought about entirely by the centromeres without any contribution of the centrosomes. The coordination of the different pollen mother cells in the plasmodial masses in organising a single spindle is difficult to visualise with the centrosomes which, if present, must necessarily be widely distributed, playing an active role. With their activity, one would expect, at best, a multipolar spindle without a regular equatorial

region. The absence of well defined poles and the regular form of the metaphase plate both point to the important role of the centromeres. The manner in which the centromeres may organise such a spindle is suggested by the observations of DARLINGTON and THOMAS (1937) on a trisomic derivative of a *Lolium-Festuca* hybrid. In this genotypically unbalanced plant, these authors found that the individual centromeres form separate spindle fibres which arrange themselves parallel with the chromosomes in an equatorial plate, without converging at the poles. It is obvious that in a plasmodial mass, the fibres formed by the centromeres in all the numerous bivalents not separated by cell walls, can be organised in a similar manner, into a single spindle, unless the chromosomes are very widely separated in which case more than one spindle of varying size, may be formed.

Having concluded that the spindles are organised without the activity of the centrosomes, it follows that the observed anaphase separation of chromosomes is also independent of polar forces which are difficult to conceive in the absence of well defined poles and without the orientation of the centrioles. The existence of polar forces which play an important role in the establishment of the metaphase configuration and in the anaphase separation of chromosomes has been postulated among others by ÖSTERGREN (1951). The importance of well defined poles in the spindle would appear to lie primarily in the grouping of the separated chromosomes into a single nucleus.

While the organisation of atypical spindles is, as pointed out earlier, a most striking feature of the abnormal meiotic behaviour, it is to be regarded meerly as an incidental outcome of the factors responsible for the failure of cell wall formation and breakdown of the division cycle. It is true that the divergent anaphase separation which these spindles give, leads to formation of nuclei with varying number of chromosomes. This unbalance, however, particularly in the case of polyploid nuclei, is not expected to result in their immediate degeneration as observed. It is far more likely that temperature treatment directly affects one or more of the mechanisms which are responsible for carrying through of the division cycle. The probable nature of the more important of these mechanisms has been discussed in recent years among others by SWANN (1957) and by HUGHES (1952).

Summary

1. Breakdown of meiotic division cycle in the plasmodial pollen mother cells of *Lolium* has been described. The plasmodia were formed in a clone following high temperature treatment of its plants.

2. Two characteristic features of meiotic sequence in the plasmodial masses are the organisation of giant metaphase plates and anaphase separation of divergent type.

3. The observations provide evidence which shows that the atypical spindles are organised exclusively through the activity of the centromeres. The lack of well defined poles in them is explained by the inactivity of centrosomes. It could be concluded that both metaphase arrangement of bivalents as well as their anaphase separation can occur independently of the polar or the centrosome forces.

4. It has been suggested that the temperature treatment affects one or more of the mechanisms responsible for carrying through of the division cycle.

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Dr. H. K. JAIN,
Division of Botany, Indian Agricultural Research Institute,
New Delhi, India

Register

Zusammengestellt von Dr. ROLAND DIETZ, Tübingen

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